cancer treatment reports

Volume 62
Number 9
September 1978
Pages 1275-1411

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

cancer treatment reports

National Cancer Institute, Arthur C. Upton, *Director*

Division of Cancer Treatment, Vincent T. DeVita, Jr., *Director*

Editorial Board

Bruce A. Chabner, *Editor-in-Chief*
Associate Editors:
Paul A. Bunn
Michael Colvin
David A. Cooney
John S. Driscoll
Marc E. Lippman
John S. Macdonald
Stephen C. Schimpff
Susan M. Sieber
John L. Ziegler

Editorial Staff

Pamela W. Jones, *Managing Editor*
Jean Griffin Baum, *Assistant Editor*
Elaine P. Beck, *Assistant Editor*
Virginia T. Ascosi, *Editorial Assistant*
Florence L. Barnes
Mary M. Davis
Deidre A. Hoehn

Advisory Board

Bernard Fisher
Emil J Freireich
Lewis W. Guiss
Simon Kramer
Faith H. Kung
LaSalle D. Leffall, Jr.
John A. Montgomery
David G. Nathan
Paul E. Neiman
Stanley E. Order
Theodore L. Phillips
Philip Rubin
Edward F. Scanlon
Stuart F. Schlossman
Joseph V. Simone

Editorial Policy

The Editorial Board will consider manuscripts dealing with all aspects of preclinical and clinical cancer treatment. These may include such topics as surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

The journal will consist of two parts. One part will consider previously unpublished manuscripts of original work under four main categories:

1. **Clinical or preclinical research.**
2. **Correspondence regarding manuscripts previously published in *Cancer Treatment Reports*. Letters on other topics will be published at the discretion of the Editorial Board.**
3. **Invited guest editorials and reviews. Unsolicited contributions will be considered on their individual merits.**
4. **Brief communications. Under this category the Editorial Board will consider short contributions which should not exceed five typewritten pages. Four types of communication will be considered in this category.**
   (a) **Ideas relating to cancer treatment.**
   (b) **Preliminary research reports.**
   (c) **Clinical case reports.**
   (d) **Brief clinical trials of novel treatments.**

All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board.

The second part of this journal will consist of supplements which may include proceedings of meetings and symposia and program information from the Division of Cancer Treatment, National Cancer Institute. Requests to publish information in *Cancer Treatment Reports* supplement will be considered by the Editorial Board.

Subscriptions

Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402. The yearly subscription rate is $38.35 domestic, with an additional $9.60 for foreign mailing. The prices vary for individual issues. Payment is required in advance; check or money order should be made payable to the Superintendent of Documents. Please specify List ID "CAN" (the identifying code for this journal) when requesting a subscription.

INSTRUCTIONS TO AUTHORS

Summary
Begin the article with a short summary.

Text
Submit an original and three copies of the manuscript, typed double spaced. Each page should begin with a new paragraph. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

Tables
Submit each table on a separate page, typed double spaced. Please use standard abbreviations; otherwise spell out.

References
Submit references typed double spaced. Follow the standard form of *Index Medicus:* author, title, name of journal, volume number, page or inclusive pages, and year. In book references give author, title of book, edition (if more than one), city and name of publisher, date of book, volume number, and pages cited. References in text should begin with number 1 and continue consecutively. References to papers presented at meetings should include author; title of paper; title, city, and date of meeting; city and name of publisher; date of publication; volume number; and pages. Papers presented at meetings but not published should be cited in a footnote rather than in the reference section.

Illustrations
Photographs and graphs preferably should be clear, glossy prints. Original art work should be done in black ink for good reproduction. Please send 2 copies of each print. Color photographs cannot be used.

Nomenclature
Names of compounds should conform to American usage. See *Chemical Abstracts* for proper nomenclature.

Abbreviations
Only standard abbreviations are acceptable. If a new or coined abbreviation is used, it must be defined when first mentioned.

Brief Reports
Manuscripts published in the Brief Reports section can be no longer than 5 double-spaced pages including references, tables, and a short summary; figures are usually omitted.

Letters to the Editor
Letters to the Editor can be no longer than 3 double-spaced text pages and can be submitted with no more than 1 table and 5 references; figures are usually omitted.

Symposia
Requests to publish the proceedings of symposia should be addressed to the Managing Editor. A copy of the agenda and a list of speakers should accompany such requests.

Submission of Manuscripts
Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, National Cancer Institute, 8300 Colesville Rd, Rm 3A05, Blair Bldg, Silver Spring, Md 20910. We invite all authors to submit with their manuscripts a short list of possible reviewers. The editorial board will take these names into consideration in selecting those who will be asked to evaluate your manuscript.

Reprints
Supplied free of charge: 250 reprints will be sent to the senior author about three weeks after publication of the article.

Contents

Cancer Treatment Reports
Vol. 62, No. 9
September 1978

Page

COMMENTARY

Effect of Primary Treatment Modality on the Metastatic Pattern of Mammary Carcinoma 1275
Anthony J. Piro and Samuel Hellman
Tufts-New England Medical Center, Boston, Massachusetts

ARTICLES

Association Between Steroid Hormone Receptors and Response Rate to Cytotoxic Chemotherapy in Metastatic Breast Cancer 1281
Joseph C. Allegra, Marc E. Lippman, E. Brad Thompson, Richard Simon, Audrey Barlock, Linda Green, Karen K. Huff, Hoan My T. Do, Susan C. Aitken, and Robert Warren
National Cancer Institute, Bethesda, Maryland

5-Fluorouracil, Methyl-CCNU, Adriamycin, and Mitomycin C in the Treatment of Advanced Gastric Cancer 1287
Paul A. Bunn, Jr, John L. Nugent, Daniel C. Ihde, Martin H. Cohen, Joyce L. Eddy, and John D. Minna
National Cancer Institute-Veterans' Administration Medical Oncology Branch, Washington, DC

Phase II Clinical Trial With High-Dose Methotrexate Therapy and Citrovorum Factor Rescue 1295
W. H. Isacoff, F. Eilber, H. Tabbarah, P. Klein, M. Dollinger, S. Lemkin, P. Sheehy, L. Cone, B. Rosenbloom, L. Sieger, and J. B. Block
University of California, Los Angeles

Pharmacokinetics of BCNU in Man: A Preliminary Study of 20 Patients 1305
Victor A. Levin, William Hoffman, and Robert J. Weinkam
University of California, San Francisco

Kinetics of Cytotoxicity of VM-26 and VP-16-213 on L1210 Leukemia and Hematopoietic Stem Cells 1313
Teresa J. Vietti, Frederick A. Valeriote, Randy Kalish, and Dean Coulter
Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Missouri

Page

Comparative Disposition of Phosphoramide Mustard and Other Cyclophosphamide Metabolites in the Mouse Using the *Salmonella*/ Mutagenesis Assay 1321
William J. Suling, Robert F. Struck, Carolyn W. Woolley, and William M. Shannon
Southern Research Institute, Birmingham, Alabama

Estramustine Phosphate in the Treatment of Advanced Malignant Melanoma 1329
Reynold Lopez, Constantine P. Karakousis, Mukund S. Didolkar, and E. Douglas Holyoke
Roswell Park Memorial Institute, Buffalo, New York

Phase I Trial of Vindesine in Patients With Advanced Cancer 1333
Violante E. Currie, Peter P. Wong, Irwin H. Krakoff, and Charles W. Young
Memorial Sloan-Kettering Cancer Center, New York, New York

Chronochemotherapy of L1210 Leukemic Mice With Cytosine Arabinoside or Cyclophosphamide 1337
William C. Rose, Mary W. Trader, W. Russell Laster, Jr, and Frank M. Schabel, Jr
Southern Research Institute, Birmingham, Alabama

BRIEF REPORTS AND PRELIMINARY COMMUNICATIONS

Combination Chemotherapy With *cis*-Dichlorodiammineplatinum(II) and Adriamycin for Testicular Cancer Refractory to Vinblastine Plus Bleomycin 1351
Lawrence H. Einhorn and Stephen D. Williams
Indiana University Medical Center, Indianapolis

Interstitial Pulmonary Fibrosis as a Complication of Prolonged Methyl-CCNU Therapy 1355
William Lee, Robert P. Moore, and Galen L. Wampler
McGuire Veterans' Administration Hospital, Richmond, Virginia

Plasma Levels and Urinary Excretion of Filterable Platinum Species Following Bolus Injection and Iv Infusion of *cis*-Dichlorodiammineplatinum(II) in Man 1359
Thomas F. Patton, Kenneth J. Himmelstein, Robert Belt, Steve J. Bannister, Larry A. Sternson, and A. J. Repta
The University of Kansas, Lawrence

Phase I Study of Neocarzinostatin in Patients With Acute Leukemia 1363
David A. Van Echo and Peter H. Wiernik
Baltimore Cancer Research Center, National Cancer Institute, Maryland

Phase II Study of Adriamycin and Bleomycin in Patients With Multiple Myeloma 1367
John M. Bennett, Robert Silber, Ediz Ezdinli, Martin Levitt, Martin Oken, Richard F. Bakemeier, John C. Bailar, and Paul P. Carbone
Eastern Cooperative Oncology Group

Page

Phase I Study of N^6-Benzyladenosine-5′-monophosphate 1371
Raphael Catane, Jerome H. Kaufman, Freda A. Nime, James T. Evans, and Arnold Mittelman
Roswell Park Memorial Institute, Buffalo, New York

Adriamycin Therapy for Advanced Ovarian Carcinoma Recurrent After Chemotherapy 1375
Susan Molloy Hubbard, Peter Barkes, and Robert C. Young
National Cancer Institute, Bethesda, Maryland

Chemo-Hormonal Therapy for Metastatic Renal Cell Carcinoma With Adriamycin, Hydroxyurea, Vinblastine, and Medroxyprogesterone Acetate 1379
S. B. Katakkar and C. R. Franks
Saskatchewan Cancer Commission, Regina, Canada

Age-Related Adriamycin Cardiotoxicity in Children 1381
Charles B. Pratt, J. Laurence Ransom, and William E. Evans
St. Jude Children's Research Hospital, Memphis, Tennessee

Treatment of Cervical Carcinoma With Adriamycin Combined With Methotrexate 1387
C. Papavasiliou, J. Pappas, D. Aravantinos, and D. Kaskarelis
Alexandra Hospital, University of Athens, Greece

Treatment of Adult Acute Lymphoblastic Leukemia With Cytosine Arabinoside, Vincristine, and Prednisone 1389
Roger D. Gingrich, James O. Armitage, and C. Patrick Burns
University of Iowa Hospitals, Iowa City

Alteration of Binding of the Supravital Dye Hoechst 33342 to Human Leukemic Cells by Adriamycin 1393
Harvey D. Preisler
Roswell Park Memorial Institute, Buffalo, New York

Invasive Thymoma Responsive to Oral Corticosteroids 1397
Judd Shellito, Janardan D. Khandekar, William P. McKeever, and Nicholas A. Vick
Evanston Hospital, Illinois

Phase II Study of Melphalan in Colorectal Carcinoma 1401
Joseph DiBenedetto, Jr, Houshang Moayeri, James T. Evans, and Arnold Mittelman
Roswell Park Memorial Institute, Buffalo, New York

LETTERS

Evaluation of a Chemotherapeutic Regimen for Primary Liver Cancer in Children 1403
Neil N. Senzer, William Terrell, and Charles B. Pratt
St. Jude Children's Research Hospital, Memphis, Tennessee

Page

Surgically Proven Pulmonary Metastases Not Demonstrated by Computed Chest Tomography 1404
E. T. Creagan, S. Frytak, P. Pairolero, R. G. Hahn, and J. R. Muhm
Mayo Clinic, Rochester, Minnesota

Prolonged Complete Remission in Malignant Fibrous Histiocytoma Treated With Chemotherapy 1405
Wilbur B. Bassett and Raymond B. Weiss
West Virginia University Hospital, Morgantown

Use of Metronidazole and Other Nitroimidazole Drugs in Cancer Therapy 1406
I. J. Stratford and G. E. Adams
Royal Marsden Hospital, Sutton, Surrey, England

Combination of Constant-Infusion 5-Fluorouracil, Methyl-CCNU, Mitomycin C, and Vincristine in Advanced Colorectal Carcinoma 1407
F. Anthony Greco, Ronald L. Richardson, Susan F. Schulman, and Robert K. Oldham
Vanderbilt University Medical Center, Nashville, Tennessee

Unusual Neuromuscular Toxicity After Treatment of Bone Sarcomas With Adriamycin, Vincristine, and Cyclophosphamide 1408
Efisio Sulis
Ospedale Oncologico "A. Businco," Cagliari, Italy

ANNOUNCEMENTS 1411

Conference on "Combined Modalities: Chemotherapy/Radiotherapy"
Conference on "The Diagnosis and Management of the Complications of Cancer"
Seminars in Oncology at Roswell Park Memorial Institute

The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1980.

COMMENTARY

Effect of Primary Treatment Modality on the Metastatic Pattern of Mammary Carcinoma [1]

Anthony J. Piro[2,*] and Samuel Hellman[3]

SUMMARY

In animal tumor systems, all three major treatment modalities, surgery, radiotherapy, and chemotherapy, may increase the incidence of metastases in the presence of circulating viable tumor cells. In breast cancer patients, selected studies can be found which report an increased incidence of metastases after surgery, radiotherapy, or chemotherapy, but these effects appear to exert little influence on overall survival. Caution is advised in using systemic therapy prior to effective primary tumor cytoreductive treatment. Clinical trials in advanced local disease should be done to test this concern. Minimal surgery, loco-regional radiotherapy, and effective adjuvant systemic therapy may result in the improved survival of patients with breast cancer with minimal functional or cosmetic impairment.

[Cancer Treat Rep 62:1275–1280, 1978]

Presently, there are three major therapeutic modalities used in the primary treatment of mammary carcinoma. Surgery and radiation therapy attempt to control the local and regional disease and adjuvant systemic therapy (chemotherapy and/or hormonal therapy) is being studied for its possible role in the eradication of subclinical distant metastases. Recent studies indicating an increased role for radiation therapy in the primary management of loco-regional disease (1–9) and for adjuvant chemotherapy in the treatment of clinically inapparent metastases (10–12) have prompted concern for possible untoward effects of each therapeutic modality. A major concern is the possible effect of such treatments on the incidence and sites of distant metastases.

Many in vivo studies have enumerated the effects of local treatment on distant metastases, often utilizing transplantable murine sarcomas or mammary carcinomas. When the transplanted "primary" tumor and/or regional lymph nodes are treated surgically or by radiation therapy, either an increase or decrease in visceral metastases may be seen, depending on the tumor system and the timing of the primary treatment (13–19). The manipulation of the immune system, for example, thymectomy (20–22), splenectomy (20), total-body irradiation (22–24), administration of antilymphocyte serum (25), and prolonged thoracic duct draining (21), increases visceral metastases due to the significant immunosuppression produced. The disturbance of the organ system at risk for metastases, for example, irradiation of the liver or lungs (26–31), surgical manipulation of the liver (32), or systemic pretreatment with cytotoxic chemotherapy (33–37), increases the incidence of such secondary deposits. The time between the intravascular tumor cell inoculation and the host manipulation is most important; pretreatment alteration followed shortly by inoculation is the most efficient metastases-producing sequence. It appears that viable tumor cells must be circulating simultaneously with or shortly after the organ system manipulation. For the oncologist, these results can be translated into clinical situations in which possible organ sites of metas-

[1]Received Feb 28, 1978; revised July 10, 1978; accepted July 19, 1978.

[2]Department of Therapeutic Radiology, Tufts-New England Medical Center, Boston, Mass.

[3]Department of Radiation Therapy, Harvard Medical School, Boston, Mass.

**Reprint requests to:* Anthony J. Piro, MD, Department of Therapeutic Radiology, Tufts-New England Medical Center, 171 Harrison Ave, Boston, Mass 02111.

tases are manipulated before, during, or shortly after viable cell seeding from the primary tumor. Consequently, for prevention of metastases, primary therapy must destroy or inactivate significant numbers of tumor cells before giving treatment which is likely to increase metastatic "take" so that few or no circulating cells are available to lodge and proliferate in these receptive organs.

Clinical Experience

For patients with breast cancer in the clinic, similar questions regarding the effect of primary therapy on metastatic potential are most germane but not easily answered. A few studies consider the incidence of distant metastases to be a consequence of primary treatment. It is important to separate the site of initial metastases from the cumulative incidence of metastases to any one site. Clearly, changes in the frequency of metastases at differing sites will occur any time metastasis to a single site is successfully prevented. Thus, apparent increases of secondaries at other sites as the initial recurrence need not signify increased incidence since the actual numbers of patients with metastases occurring at these sites may remain the same or indeed even decrease.

The scar recurrence seen after surgery has been attributed to local cell implantation, but many surgeons feel it may be caused by blood-borne seeding in a surgically damaged tissue. Evidence for the latter is the occasional recurrence of tumor in the healed scar of a graft donor site (38). Increased metastases in an incision have been demonstrated in animal models (39,40).

In selected patients treated with different combinations of surgery and radiation, one study reported that postoperative radiotherapy increased ipsilateral pulmonary metastases (41). However, this observation was not substantiated by Chu et al (42) who found no increase in ipsilateral skin or pulmonary metastases in a large number of postoperatively irradiated patients. The suggestion of increased metastases has been heightened by the recent compilation by Stjernswärd, proposing that postmastectomy radiation decreased survival (43). He suggests that low lymphocyte counts and immune deficiency following postoperative radiotherapy caused the lowered survival. A number of subsequent reports (44–53) indicate that Stjernswärd's conclusions are incorrect and careful data analysis does not indicate a statistically significant survival reduction. Furthermore, the studies quoted may not represent optimal radiation therapy, since there are two recent studies showing a survival advantage. A prospective randomized trial using modern supervoltage techniques by de Schryver (54) compared preoperative or postoperative radiotherapy with no irradiation following modified radical mastectomy. The expected decrease in local recurrence was seen in both irradiated groups, but more importantly, the initial data suggest a survival advantage in the irradiated groups concomitant with a decrease in distant metastases. Høst and Brennhovd have recently reported a controlled randomized trial which shows a statistically significant increase in survival for postoperatively irradiated stage II patients (55).

Increased distant metastases in patients receiving postoperative radiation therapy are reported in the National Surgical Adjuvant Breast Project study of postoperative irradiation or thioTEPA as adjuncts to radical mastectomy versus surgery alone (56). Considering *all* relapses, the incidence of pulmonary metastases appeared increased in both the thioTEPA-treated (27%) and the irradiated (28%) groups, as compared to the control group (15%). Most importantly, all three groups had approximately a 50% failure rate at 5 years, indicating no difference in the overall incidence of recurrent disease. The higher proportion of initial distant failure in the postoperative radiation therapy group was stressed (77%) as opposed to control patients (58%) and thioTEPA-treated patients (61%). This difference is spurious because the radiation therapy was successful in markedly reducing loco-regional recurrence, and the study scored only the *initial* site of relapse. Local recurrence is frequently a harbinger of distant disease, which may be detected either simultaneously with or soon after local recurrence (57–59). Consequently, if patients destined to have both local and distant failure have local recurrence prevented by radiotherapy, the first site of failure must be distant, but the overall number of patients who eventually fail remains the same. In the Manchester trial conducted by Paterson et al, a slight increase in the early appearance of hepatic metastases in a subgroup of patients receiving immediate postoperative irradiation was seen as compared to the "watched" controls (60), but this difference was insignificant at 7 years (61).

Earlier adjuvant chemotherapy trials, including the above National Surgical Adjuvant Breast Project study, have been thoroughly reviewed by Tormey (62); several reports indicate the initial sites of relapse. In three studies (56,63,64), a decrease in local recurrence in the treated group was seen; more osseous metastases were noted in the treated group in two reports (65,66) and increased pulmonary metastases were noted in one report (56) as we previously discussed. The Milan trial by Bonadonna with combination chemotherapy has reported a

decrease in all sites of metastases in treated patients (12,67). For proper evaluation, the cumulative incidence of metastases should be scored at each site and, most importantly, the proportion of patients who fail from whatever cause must be carefully ascertained. Current studies of adjuvant chemotherapy have been impressive in apparently extending the relapse-free survival (10–12). If a significant number of these patients eventually have recurrence, it will be of paramount importance to document the cumulative incidence of metastases at each site as well as the site of initial metastasis.

There have been many trials of the use of ovarian castration as an adjuvant to mastectomy in early breast cancer (61,68–71). An increase in disease-free intervals is noted with adjunctive oophorectomy, but subsequent hormonal manipulation in the control patients appears to equalize overall survival in the two groups. Only the Manchester study (61,68) details sites of metastases, reporting a slight decrease in both local and distant metastases in patients who had adjunctive oophorectomy. Clinical trials are now in progress to assess the role of hormonal therapy (antiestrogens) in conjunction with chemotherapy as adjuvants to mastectomy.

Patterns of breast cancer recurrence have been recently studied in a general way without specific correlation with primary therapy (72–74).

The correlation of primary therapy with the site of initial metastasis and the cumulative incidence may afford important clues to the next therapeutic step in the overall treatment. An obvious requirement of such correlations is regular and complete patient followup with consistent and careful documentation of time and site of first relapse.

Rationale for Current Therapy

To develop an appropriate treatment schema, general principles learned from the laboratory and the clinic must be considered. Experimental studies indicate that the number of distant metastases can be increased by pretreatment of the host with any of the currently used therapeutic modalities (surgery, radiation, or cytotoxic chemotherapy). This occurs only when the primary tumor is *not* treated or when tumor cells are actively infused (not a clinical concern). Conversely, the incidence of metastases should be reduced if the host receives an effective systemic adjuvant treatment when, and possibly *only* when, the primary tumor has been or is being effectively treated, thus providing little or no opportunity for viable cell seeding.

There are clinical examples of such therapeutic successes. Adjuvant chemotherapy and whole-body irradiation are effective in treating micrometastases in Ewing's sarcoma (75,76). Brain irradiation and intrathecal methotrexate markedly reduce central nervous system metastases in leukemic patients achieving complete remission by systemic chemotherapy (77,78). This latter example is important, for even if such treatments were to predispose the host to leukemic cell seeding, there would be no viable circulating leukemia cells, and, therefore, these treatments are effective in destroying micrometastases within the central nervous system. Radiation is frequently administered postoperatively to the tumor bed in Wilms' tumor concomitantly with actinomycin D administration. Although such treatment irradiates a significant portion of the liver and lower lung(s), it has not been associated with an increase in metastases to these organs but rather with an overall survival increment (79). These patients also benefit from adjuvant chemotherapy because of the consequent prevention of pulmonary metastases. Preoperative and/or postoperative irradiation in rectal carcinomas (80–82), head and neck tumors (83–87), and soft tissue sarcomas (88) result in decreased local recurrence, no evidence of increased distant metastases, and a concomitant survival advantage.

Such studies indicate that similar multimodality therapy can be given for carcinoma of the breast. However, care should be taken when treatment designed to prevent the growth of distant metastases precedes effective cytoreductive therapy to the primary tumor. It is possible that treatment of primary carcinoma of the breast with systemic chemotherapy before effective local tumor therapy may be hazardous, depending on the relative effects of tumor cytotoxicity on the one hand and increased tumor cell "take" on the other hand. A primary treatment regimen of surgery and/or radiation therapy, followed by or concomitant with adjuvant systemic chemotherapy, would appear to obviate this possibility. In other diseases where local cytoreductive therapy has been employed prior to systemic treatment, significant improvement in survival has been obtained.

Several studies employing systemic chemotherapy followed by local radiation therapy and subsequent maintenance chemotherapy have shown encouraging preliminary results (89–91). However, longer followup of these patients is needed, and comparative clinical trials of alternate sequences of the different modalities should be conducted with consideration of sites of recurrence as well as disease-free and overall survival to test the concern of systemic treatment prior to local therapy.

Whether radiation therapy or surgery should be the primary modality to treat the breast tumor

should be determined by the efficacy of these treatments in the prevention of local recurrence and the extent to which each causes functional or cosmetic impairment. To date, the results with radiation therapy following excisional biopsy for breast cancer appear encouraging (1–9). In most studies, such treatment seems to neither increase nor diminish the incidence of distant metastases, which hopefully will be prevented by adjuvant systemic therapy. Peters (8) reported fewer skeletal and pulmonary metastases in women treated by excision and radiation as compared to matched mastectomy and radiation-treated patients. The combination of minimal surgery (tumorectomy), loco-regional radiation therapy, and adjuvant chemotherapy may result in increased survival of patients with breast cancer with minimal or no functional or cosmetic impairment.

REFERENCES

1. PETERS MV. Cutting the "Gordian knot" in early breast cancer. Ann R Coll Phys Surg Canada 8:186–192, 1975.
2. WEBER E, and HELLMAN S. Radiation as primary treatment for local control of breast carcinoma. JAMA 234:608–611, 1975.
3. LEVENE MB, HARRIS JR, and HELLMAN S. Treatment of carcinoma of the breast by radiation therapy. Cancer 39:2840–2845, 1977.
4. PROSNITZ LR, GOLDENBERG IS, PACKARD RA, ET AL. Radiation therapy as initial treatment for early stage cancer of the breast without mastectomy. Cancer 39:917–923, 1977.
5. PIERQUIN B, BAILLET F, and WILSON JF. Radiation therapy in the management of primary breast cancer. AJR 127:645–648, 1976.
6. SPITALIER J, BRANDONE H, AYME Y, ET AL. Cesiumtherapy of breast cancer. A five-year report on 400 consecutive patients. Int J Radiat Oncol Biol Phys 2:231–235, 1977.
7. HELLMAN S. The place of radiation in the definitive management of carcinoma of the breast. Int J Radiat Oncol Biol Phys 2:369–370, 1977.
8. PETERS MV. Wedge resection with or without radiation in early breast cancer. Int J Radiat Oncol Biol Phys 2:1151–1156, 1977.
9. BROWN GS, KRAMER S, BRADY L, ET AL. Primary irradiation of stage I and stage II adenocarcinoma of the breast. Int J Radiat Oncol Biol Phys 2:1145–1148, 1977.
10. FISHER B, CARBONE P, ECONOMOU SG, ET AL. L-Phenylalanine mustard (L-PAM) in the management of primary breast cancer. A report of early findings. N Engl J Med 292:117–122, 1975.
11. BONADONNA G, BRUSAMOLINO E, VALAGUSSA P, ET AL. Combination chemotherapy as an adjuvant treatment in operable breast cancer. N Engl J Med 294:405–410, 1976.
12. BONADONNA G. Present status of CMF adjuvant therapy in operable breast cancer. Int J Radiat Oncol Biol Phys 2:237–240, 1977.
13. KAPLAN HS, and MURPHY FD. The effect of local roentgen irradiation on the biological behavior of a transplantable mouse carcinoma. I. Increased frequency of pulmonary metastases. J Natl Cancer Inst 9:407–413, 1949.
14. FISHER B, and FISHER ER. Metastases of cancer cells. *In* Methods in Cancer Research (Bush M, ed). New York, Academic Press, 1967, vol 1, p 243.
15. CRILE G. The effect on metastasis of removing or irradiating regional nodes of mice. Surg Gynecol Obstet 126:1270–1272, 1968.
16. CRILE G, and DEODHAR SD. Role of preoperative irradiation in prolonging concomitant immunity and preventing metastasis in mice. Cancer 27:629–634, 1971.
17. SUIT HD, SEDLACEK RS, and GILLETTE EL. Examination for a correlation between probabilities of development of distant metastasis and of local recurrence. Radiology 95:189–194, 1970.
18. FISHER B, and FISHER ER. Studies concerning the regional lymph node in cancer. II. Maintenance of immunity. Cancer 29:1496–1500, 1972.
19. MCCREDIE JA, INCH WR, and COWIE HC. Effect of excision or local radiotherapy to a tumor and its regional nodes on metastases. Cancer 31:983–987, 1971.
20. KIM V. Metastasizing mammary carcinomas in rats: induction and study of their immunogenicity. Science 167:72–74, 1970.
21. ECCLES SA, and ALEXANDER P. Macrophage content of tumours in relation to metastatic spread and host immune reaction. Nature 250:667–669, 1974.
22. SUURKULA M, and BOERYD B. Tumour metastases in mice with reduced immune reactivity. II. Studies with a highly antigenic MCA induced sarcoma in thymectomized and/or sublethally irradiated C57BL/6J mice. Int J Cancer 14:633–641, 1974.
23. BARNES DWH, FORD CE, ILBERY PLT, ET AL. Tissue transplantation in the radiation chimera. J Cell Comput Physiol (suppl 1) 50:123–138, 1957.
24. FELDMAN M, GLOBERSON A, and YAFFE D. Immunological properties of chemically induced sarcomas. Acta Un Int Cancr 19:65–72, 1963.
25. FISHER B, SOLIMAN O, and FISHER ER. Effect of antilymphocyte serum on parameters of tumor growth in a syngeneic tumor-host system. Proc Soc Exp Biol Med 131:16–18, 1969.
26. DAO TL, and YOGO H. Enhancement of pulmonary metastases by X-irradiation in rats bearing mammary cancer. Cancer 20:2020–2025, 1967.
27. FISHER ER, and FISHER B. Effects of X-irradiation on parameters of tumor growth, histology, and ultrastructure. Cancer 24:39–55, 1969.
28. BROWN JM. The effect of lung irradiation on the incidence of pulmonary metastases in mice. Br J Radiol 46:613–618, 1973.
29. WITHERS HR, and MILAS L. Influence of preirradiation of lung on development of artificial pulmonary metastases of fibrosarcoma in mice. Cancer Res 33:1931–1936, 1973.
30. SHAEFFER J, EL-MAHDI AM, and CONSTABLE WC. Radiation control of microscopic pulmonary metastases in C3H mice. Cancer 32:346–351, 1973.
31. THOMPSON SC. Tumour colony growth in the irradiated mouse lung. Br J Cancer 30:337–341, 1974.
32. FISHER B, and FISHER ER. Experimental studies of factors influencing hepatic metastases. III. Effect of surgical trauma with special reference to liver injury. Ann Surg 150:731–744, 1959.
33. KONDO T, and MOORE GE. Production of metastases by treatment with carcinostatic agents. I. Effects of carcinostatic agents on the host. Cancer Res 21:1396–1399, 1961.
34. FISHER B, and FISHER ER. Effect of experimental perfusion upon the biologic and pathologic behavior of tumor. Surgery 56:651–662, 1964.
35. SUGARBAKER EV, COHEN AM, and KETCHAM AS. Facilitated metastatic distribution of the Walker 256 tumor in Sprague Dawley rats with hydrocortisone and/or cyclophosphamide. J Surg Oncol 2:277–289, 1970.

36. DEODHAR SD. Enhancement of metastases by L-asparaginase in a mouse tumor system. J Reticuloendothel Soc 10:212–222, 1971.
37. CARMEL RJ, and BROWN JM. The effect of cyclophosphamide and other drugs on the incidence of pulmonary metastases in mice. Cancer Res 37:145–151, 1977.
38. JEWELL WR, and ROMSDAHL MM. Recurrent malignant disease in operative wounds not due to surgical implantation from the resected tumor. Surgery 58:806–809, 1965.
39. ALEXANDER JW, and ALTEMEIER WA. Susceptibility of injured tissues to hematogenous metastases. Ann Surg 159:933–944, 1964.
40. FISHER B, FISHER ER, and FEDUSKA N. Trauma and the localization of tumor cells. Cancer 20:23–30, 1967.
41. DAO TC, and KOVARIC J. Incidence of pulmonary and skin metastases in women with breast cancer who received postoperative irradiation. Surgery 52:203–212, 1962.
42. CHU FCH, LUCAS JC, JR, FARROW JH, ET AL. Does prophylactic radiation therapy given for cancer of the breast predispose to metastasis? AJR 99:987–994, 1967.
43. STJERNSWÄRD J. Decreased survival related to irradiation postoperatively in early operable breast cancer. Lancet 2(7892):1285–1286, 1974.
44. PAPATESTAS AE, and KARK AE. Peripheral lymphocyte counts in breast cancer: an index of immune competence. Cancer 34:2014–2017, 1974.
45. LEVITT SH, and MCHUGH RB. Early breast cancer and postoperative irradiation. Lancet 2(7947):1258–1259, 1975.
46. HALNAN KE. Letter: Postoperative radiotherapy and breast cancer. Lancet 1(7974):1401, 1976.
47. BLOMGREN H, BERG R, WASSERMAN J, ET AL. Effect of radiotherapy on blood lymphocyte population in mammary carcinoma. Int J Radiat Oncol Biol Phys 1:177–188, 1976.
48. GLAS U, WASSERMAN J, BLOMGREN H, ET AL. Lymphopenia and metastatic breast cancer patients with and without radiation therapy. Int J Radiat Oncol Biol Phys 1:189–195, 1976.
49. ALEXANDER P. The bogey of the immunosuppressive action of local radiotherapy. Int J Radiat Oncol Biol Phys 1:369–371, 1976.
50. BRADY LW, FLETCHER GH, and LEVITT SH. Cancer of the breast: the role of radiation therapy after mastectomy. Cancer 39:2868–2874, 1977.
51. LEVITT SH, and MCHUGH RB. Radiotherapy in the postoperative treatment of operable cancer of the breast. I. Critique of the clinical and biometric aspects of the trials. Cancer 39:924–932, 1977.
52. LEVITT SH, MCHUGH RB, and SONG CW. Radiotherapy in the postoperative treatment of operable cancer of the breast. II. A re-examination of Stjernswärd's application of the Mantel-Haenszel statistical method. Evaluation of the effect of the radiation on immune response and suggestions for postoperative radiotherapy. Cancer 39:933–940, 1977.
53. FLETCHER GH. Reflections on breast cancer. Int J Radiat Oncol Biol Phys 1:769–779, 1976.
54. DE SCHRYVER A. The Stockholm breast cancer trial: preliminary report of a randomized study concerning the value of pre-operative or post-operative radiotherapy in operable disease. Int J Radiat Oncol Biol Phys 1:601–609, 1976.
55. HØST H, and BRENNHOVD IO. The effect of post-operative radiotherapy in breast cancer. Int J Radiat Oncol Biol Phys 2:1061–1067, 1977.
56. FISHER B, SLACK NH, CAVANAUGH PJ, ET AL. Postoperative radiotherapy in the treatment of breast cancer. Results of the NSABP clinical trial. Ann Surg 172:711–732, 1970.
57. DONEGAN WL, PEREZ-MESA CM, and WATSON FR. A biostatistical study of locally recurrent breast carcinoma. Surg Gynecol Obstet 122:529–540, 1966.
58. SPRATT JS. Locally recurrent cancer after radical mastectomy. Cancer 20:1051–1053, 1967.
59. BRUCE J, CARTER DC, and FRASER J. Patterns of recurrent disease in breast cancer. Lancet 1:433–435, 1970.
60. PATERSON R, and RUSSELL MH. Clinical trials in malignant disease. III. Breast cancer: evaluation of post-operative radiotherapy. J Fac Radiol 10:175–180, 1959.
61. COLE M. The place of radiotherapy in the management of early breast cancer. A report of two clinical trials. Br J Surg 51:216–220, 1964.
62. TORMEY DC. Combined chemotherapy and surgery in breast cancer: a review. Cancer 36:881–892, 1975.
63. RIECHE K, BERNDT H, and PRAHL B. Continuous postoperative treatment with cyclophosphamide in breast carcinoma—a randomized clinical study. Arch Geschiwulstforsch 40:349–354, 1972.
64. DONEGAN WL. Extended surgical adjuvant thiotepa for mammary carcinoma. Arch Surg 109:187–192, 1974.
65. FISHER G, RAVDIN RG, AUSMAN RK, ET AL. Surgical adjuvant chemotherapy in cancer of the breast—results of a decade of cooperative investigation. Ann Surg 168:337–356, 1968.
66. FINNEY MB. Adjuvant chemotherapy in the radical treatment of carcinoma of the breast—a clinical trial. AJR 111:137–141, 1971.
67. BONADONNA G. Letter: Local-regional recurrence after mastectomy. Lancet 2(7979):260–261, 1976.
68. PATERSON R, and RUSSELL MH. Clinical trials in malignant disease. II. Breast cancer: value of irradiation of the ovaries. J Fac Radiol 10:130–133, 1959.
69. KENNEDY BJ, MIELKE PW, JR, and FORTUNY IE. Therapeutic castration versus prophylactic castration in breast cancer. Surg Gynecol Obstet 118:524–540, 1964.
70. NISSEN-MEYER R. The role of prophylactic castration in the therapy of human mammary cancer. Eur J Cancer 3:395–403, 1967.
71. NEVINNY HB, NEVINNY D, ROSOFF CB, ET AL. Prophylactic oophorectomy in breast cancer therapy. Am J Surg 117:531–536, 1969.
72. VIADANA E, COTTER R, PICKREN JW, ET AL. An autopsy study of metastatic sites of breast cancer. Cancer Res 33:179–181, 1973.
73. SMALLEY RV. Five clinical patterns of recurrence of ca of the breast. Proc Am Assoc Cancer Res and ASCO 17:172, 1976.
74. HENDERSON IC, LOKICH J, MAYER R, ET AL. Carcinoembryonic antigen (CEA) levels in metastatic breast cancer: quantitative correlation with pattern of metastases. Proc Am Assoc Cancer Res and ASCO 17:202, 1976.
75. POMEROY TC, and JOHNSON RE. Combined modality therapy of Ewing's sarcoma. Cancer 35:36–47, 1975.
76. JENKIN RDT, RIDER WD, and SONLEY MJ. Ewing's sarcoma. Adjuvant total body irradiation, cyclophosphamide, and vincristine. Int J Radiat Oncol Biol Phys 1:407–413, 1976.
77. AUR RJA, SIMONE JV, HUSTU HO, ET AL. A comparative study of central nervous system irradiation and intensive chemotherapy early in remission of childhood acute lymphocytic leukemia. Cancer 29:381–391, 1972.
78. DRITSCHILO A, CASSADY JR, CAMITTA B, ET AL. The role of irradiation in central nervous system treatment and prophylaxis for acute lymphoblastic leukemia. Cancer 37:2729–2735, 1976.
79. CASSADY JR, TEFFT M, FILLER RM, ET AL. Considerations in the radiation therapy of Wilms' tumor. Cancer 32:598–608, 1973.
80. KLIGERMAN MM, URDANETA N, KNOWLTON A, ET AL. Preoperative irradiation of rectosigmoid carcinoma including its regional lymph nodes. AJR 114:498–503, 1972.
81. ROSWIT B, HIGGINS GA, JR, and KEEHN RJ. Preoperative

irradiation for carcinoma of the rectum and rectosigmoid colon: report of a national Veterans Administration randomized study. Cancer 35:1597–1602, 1975.

82. STEVENS KR, JR, ALLEN CV, and FLETCHER WS. Preoperative radiotherapy for adenocarcinoma of the rectosigmoid. Cancer 37:2866–2874, 1976.
83. WANG CC, SCHULZ MD, and MILLER D. Combined radiation therapy and surgery for carcinoma of the supraglottis and pyriform sinus. Am J Surg 124:551–554, 1972.
84. LINDBERG RD, JESSE RH, and FLETCHER GH. Radiotherapy—before and after surgery. *In* Neoplasia of the Head and Neck. Chicago, Year Book Medical Publishers, 1974, pp 47–48.
85. HENSCHKE UK, FRAZELL EL, HILARIS BS, ET AL. Value of preoperative X-ray therapy as an adjunct to radical neck dissection. Radiology 86:450–453, 1966.
86. OGURA JH, and BILLER HF. Pre-operative irradiation for laryngeal and laryngopharyngeal cancers. Laryngoscope 80:802–810, 1970.
87. NORTHROP M, FLETCHER GH, JESSE RH, ET AL. Evolution of neck disease in patients with primary squamous cell carcinoma of the oral tongue, floor of mouth, and palatine arch, and clinically positive neck nodes neither fixed nor bilateral. Cancer 29:23–30, 1972.
88. SUIT HD, RUSSELL WO, and MARTIN RG. Sarcoma of soft tissue: clinical and histopathologic parameters and response to treatment. Cancer 35:1478–1483, 1975.
89. DE LENA M, ZUCALI R, VIGANOTTI G, ET AL. Combined chemotherapy (CT)—radiotherapy (RT) in primary inoperable (T3b–T4) breast cancer. Proc Am Assoc Cancer Res and ASCO 18:356, 1977.
90. MONTAGUE ED. Radiation management of advanced breast cancer. Int J Radiat Oncol Biol Phys 4:305–307, 1978.
91. HORTOBAGYI G, BLUMENSCHEIN G, TASHIMA C, ET AL. Multidisciplinary treatment of locally advanced (stage III) breast cancer. Proc Am Assoc Cancer Res and ASCO 19:361, 1978.

Association Between Steroid Hormone Receptors and Response Rate to Cytotoxic Chemotherapy in Metastatic Breast Cancer [1]

Joseph C. Allegra, Marc E. Lippman,* E. Brad Thompson, Richard Simon, Audrey Barlock, Linda Green, Karen K. Huff, Hoan My T. Do, Susan C. Aitken, and Robert Warren [2,3]

SUMMARY

The influence of steroid hormone receptors on response rate to cytotoxic chemotherapy in 70 patients with metastatic breast cancer was determined in a retrospective study. We have previously reported that 34 of 45 patients with tumors containing low or absent estrogen-receptor values had objective responses to chemotherapy while three of 25 patients with positive estrogen-receptor tumors responded. In the present study, 22 of 34 patients with low or absent progesterone-receptor tumors had an objective response to cytotoxic chemotherapy, while none of eight patients with a positive progesterone-receptor tumor responded ($P < 0.05$). Patients having tumors with a negative estrogen receptor and a negative progesterone receptor had a response rate of 88% (21 of 24 patients). There were three patients whose tumors were estrogen-receptor negative but progesterone-receptor positive; none had a response to chemotherapy. Chemotherapy response was not associated with the presence or absence of either androgen or glucocorticoid receptor. We conclude that progesterone-receptor values in addition to estrogen-receptor status may prove to be important correlates of response to cytotoxic chemotherapy in metastatic breast cancer. Androgen- and glucocorticoid-receptor analyses are not helpful in predicting response to chemotherapy.

[Cancer Treat Rep 62:1281–1286, 1978]

It is now well-established that knowledge of estrogen-receptor status is useful in predicting response to endocrine therapy in metastatic breast cancer. Approximately two thirds of patients whose tumors are estrogen-receptor positive will respond to endocrine therapy whereas < 10% whose tumors are estrogen-receptor negative have an objective response (1). We have recently reported on a new role for the estrogen receptor in predicting response to cytotoxic chemotherapy (2). Patients whose tumors were estrogen-receptor negative responded to cytotoxic chemotherapy at a rate of 76% (34 of 45 patients), while those whose tumors were estrogen-receptor positive responded at a rate of only 12% (three of 25 patients).

The present study extends this work and analyzes response rates to cytotoxic chemotherapy as a function of the progesterone, androgen, and glucocorticoid receptors.

[1]Received Apr 14, 1978; revised July 13, 1978; accepted July 20, 1978.

[2]Division of Cancer Treatment, National Cancer Institute, Bethesda, Md (*J. C. Allegra, M. E. Lippman, E. B. Thompson, R. Simon, A. Barlock, K. K. Huff, H. M. T. Do, S. C. Aitken,* and *R. Warren*), and the Department of Oncology, Howard University, Washington, DC (*L. Green*).

[3]We wish to thank the many collaborating physicians who submitted clinical material for analysis and allowed examination of their patients' records including: Drs. Alford, Blom, Borssuck, Camalier, Casey, Egan, Funderburk, Gold, Hiltabidle, Isaacson, Jonas, Jones, Kiernan, Kim, Kirson, Kison, Kleinberg, MacDonald, Mitchell, Moore, Palmer, Perlin, Schein, Scully, Thistlethwaite, Tsangaris, Watkins, and Wherry. We would also like to thank C. Baughman and V. Tinley for their assistance in reviewing the patient records at the George Washington University Medical Center, M. Allegra for her assistance in reviewing the records at Georgetown University, P. De Steffano for assistance in data management and computerization, and Dr. D. C. Tormey (formerly of the Medicine Branch), Dr. R. C. Young, and other members of the Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, for their assistance with this study.

**Reprint requests to:* Marc E. Lippman, MD, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 6B02, National Institutes of Health, Bethesda, Md 20014.

MATERIALS AND METHODS

Receptor Assays

Biopsy specimens of metastatic or inoperable localized breast cancer were trimmed of excess fat and nontumorous tissue and a portion was submitted for confirming pathology in all cases. Samples for steroid hormone-receptor assay were kept on ice and then frozen in liquid nitrogen within 20 minutes. Steroid hormone assays were performed as previously described (3,4). When sufficient sample permitted, Scatchard analyses were performed to quantify the number of binding sites; otherwise, assays were performed twice at one or two concentrations of steroid hormone chosen to exceed several times the expected equilibrium dissociation constant. Data were analyzed using computer-assisted methods (5). A positive androgen- or glucocorticoid-receptor assay was taken to be $\geq$ 10 femtomols of [^{3}H]dihydrotestosterone or [^{3}H]dexamethasone binding per mg of cytoplasmic protein. A positive progesterone receptor was taken to be $\geq$ 20 femtomols of [^{3}H]R5020 binding per mg of cytoplasmic protein.

Patients

Seventy patients with metastatic or surgically unresectable primary breast cancer had one or more steroid hormone-receptor assays performed immediately prior to the institution of a cytotoxic chemotherapy regimen. Generally speaking, the selection of patients for a chemotherapy trial as opposed to an endocrine manipulation was not done as a result of the steroid hormone-receptor assay. In all patients, assessment of response was performed using standardized response criteria (6). In brief, complete response required the disappearance of all measurable disease including healing of all bone lesions and a return of the patients to a premorbid performance status. Partial response required a shrinkage of $\geq$ 50% of all measurable disease. No new lesions could appear and no growth could be observed in a pre-existent lesion. Although a given lesion might not regress by 50%, regression averaged over all lesions had to be $\geq$ 50%. For purposes of this study, no patient was classified as achieving a partial response unless improvement was maintained for $\geq$ 2 months. Objective response rate, as used in the figures and tables, is the sum of complete and partial response rates only. Any patient not achieving this degree of improvement was termed a nonresponder. All patient records were examined and assessed by investigators (Drs. Allegra, Warren, and Green and Ms. Barlock) unaware of steroid hormone-receptor results.

Statistical Analyses

Comparisons of proportions were performed by the contingency chi-square test with continuity correction. Comparison of continuous or ordered polychotomous distributions was performed by the Wilcoxon rank-sum test adjusted for ties (7). This test requires no distributional assumptions of the data as would a Student's t test.

RESULTS

Seventy patients had at least one steroid hormone-receptor assay performed on a sample obtained immediately prior to a chemotherapy trial. The characteristics of these 70 patients are described in detail in a previous publication (2). Briefly, the 70 patients had a mean age of 51 years, 75% were postmenopausal, and they had a mean Karnofsky performance score of approximately 90. Only approximately 20% of the group had received any prior therapy.

Of these 70 patients, 42 had a progesterone-receptor assay performed, 43 had an androgen-receptor assay performed, and 39 had a glucocorticoid-receptor assay performed.

Progesterone Receptor

Of the 42 patients who had a progesterone-receptor assay performed on their tumor, eight were progesterone-receptor positive and 34 were progesterone-receptor negative. The characteristics of these two groups are listed in table 1. The progesterone-receptor positive and negative patients are essentially identical with respect to age, menopausal status, Karnofsky performance score, disease-free interval, number of sites involved with metastatic disease, and presence of visceral involvement. The mean progesterone-receptor value for the progesterone-receptor positive tumors was 35 femtomols/mg of cytoplasmic protein compared to 3 femtomols for the progesterone-receptor negative tumors. Thirty-eight percent of the patients in the progesterone-receptor positive group had received prior endocrine therapy or chemotherapy compared to 13% of the progesterone-receptor negative group. This difference is not statistically significant. With regard to chemotherapy evaluated, 62% of patients with a positive progesterone-receptor assay received a chemotherapy combination regimen containing adriamycin compared to 72% in the progesterone-receptor negative group. All of the patients were treated in a similar manner independent of receptor status, and the time from first recurrence to this chemotherapy trial was also similar for the proges-

TABLE 1.—Characteristics of patients treated with cytotoxic chemotherapy as a function of progesterone-receptor status

	Progesterone-receptor positive	Progesterone-receptor negative	*P* value (progesterone-receptor positive vs progesterone-receptor negative)
No. of patients treated	8	34	
Age (mean ± SD)	49 ± 10	52 ± 12	$P > 0.1$
No. of patients—			
Premenopausal	1 (12%)	8 (24%)	$P > 0.1$
Postmenopausal	7 (88%)	26 (76%)	
Karnofsky score (mean ± SD)	88 ± 2	92 ± 1	$P > 0.1$
Disease-free interval in mos (median)	10	11	$P > 0.1$
No. of patients with visceral disease	5 (62%)	13 (38%)	$P > 0.1$
No. of sites involved			
1	1 (12%)	15 (44%)	
2	3 (38%)	7 (21%)	$P > 0.1$
≥ 3	4 (50%)	12 (35%)	

terone-receptor positive and negative groups. The distribution of the progesterone-receptor values and its association with response rate to cytotoxic chemotherapy are shown in figure 1. As seen in table 2, none of eight patients whose tumors contained progesterone receptor in excess of 20 femtomols/mg of cytoplasmic protein responded objectively to chemotherapy whereas 22 of 34 (64%) patients whose tumors lacked progesterone receptor responded ($P < 0.05$). At the present time, we are unable to define with certainty a progesterone-receptor value cutoff for chemotherapy response more helpful than the 20-femtomol value which was chosen arbitrarily. Even without regard to the cutoff value of positivity, the distribution of progesterone-receptor values for responders differs significantly by Wilcoxon rank-sum analysis from the corresponding distribution for nonresponders ($P < 0.01$). Thus, there is a highly significant correlation between chemotherapy response rate and quantitative progesterone-receptor analysis. Response rate to chemotherapy as a function of both the estrogen and progesterone receptors is shown in table 3. The response rate in patients who are estrogen-receptor positive is very low, an observation which we have previously reported (2). This response rate does not change as a function of the presence or absence of a progesterone receptor. Progesterone receptor, however, does appear to affect the response rate of the

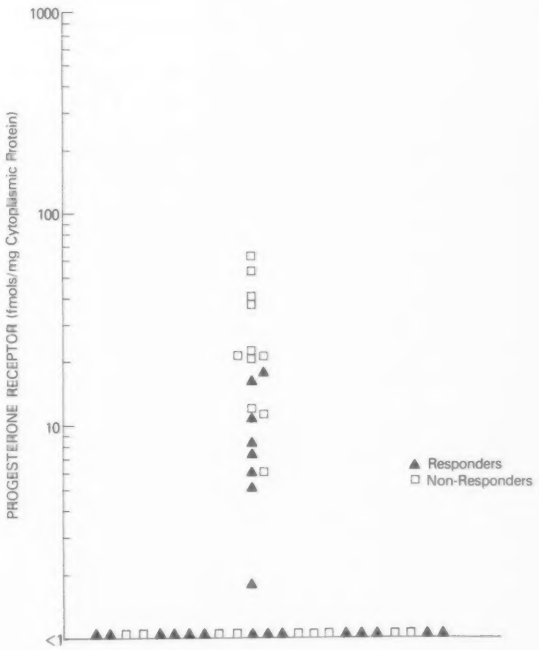

FIGURE 1.—Influence of progesterone-receptor status on objective response rate to cytotoxic chemotherapy in metastatic breast cancer. Objective response is the sum of complete + partial responders.

TABLE 2.—Objective response rate to cytotoxic chemotherapy as a function of progesterone-receptor status

Status	Response rate	
Progesterone-receptor positive	0 (0 of 8 patients)	$P < 0.01$
Progesterone-receptor negative	64% (22 of 34 patients)	

patients whose tumors lack estrogen receptor. Patients who are estrogen- and progesterone-receptor negative have a response rate of 88% (21 of 24 patients). Unfortunately, there are only three patients who are estrogen-receptor negative but progesterone-receptor positive. None of these patients responded, but the group is too small to perform a meaningful statistical test.

Androgen Receptor

Of the 43 patients who had an androgen-receptor assay performed on their tumor, 11 were androgen-receptor positive and 32 were androgen-receptor negative. The distribution of androgen-receptor values and its association with response rate to cytotoxic chemotherapy are shown in figure 2. Five of 11 (45%) patients whose tumors were androgen-receptor positive had an objective response to chemotherapy and 16 of 32 (50%) patients whose tumors had low androgen-receptor values responded ($P > 0.1$). Even without regard to the cutoff value of positivity for the androgen receptor, the distribution of receptor values for responders did not differ significantly by Wilcoxon rank-sum analysis from the corresponding distribution for nonresponders. The response rate to cytotoxic chemotherapy as a function of both estrogen and androgen receptor is shown in table 4. The presence or absence of an androgen receptor does not alter the response rate in patients who are estrogen-receptor negative. No difference between the androgen-receptor positive and negative groups with regard to age, menopausal status, Karnofsky performance score, disease-free interval, number of sites involved with metastatic disease, or presence of visceral involvement could be identified, which explained why androgen

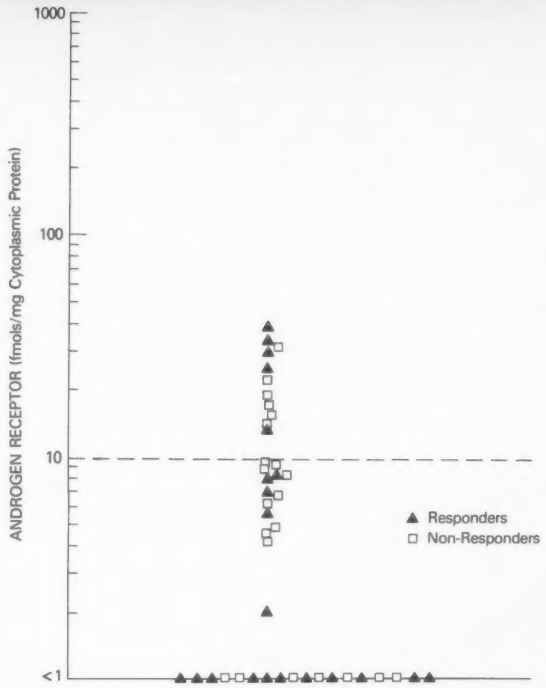

FIGURE 2.—Influence of androgen-receptor status on objective response rate to cytotoxic chemotherapy in metastatic breast cancer. Objective response is the sum of complete + partial responders.

receptor did not correlate with response to cytotoxic chemotherapy.

Glucocorticoid Receptor

Of the 39 patients who had a glucocorticoid-receptor assay performed on their tumors, 31 were glucocorticoid-receptor positive and eight were glucocorticoid-receptor negative. The distribution of the glucocorticoid-receptor values and its association with response rate to cytotoxic chemotherapy are shown in figure 3. Fourteen of 31 (45%) patients whose tumors were glucocorticoid-receptor positive responded to chemotherapy while five of eight (62%) patients whose tumors lacked glucocorticoid receptor responded ($P > 0.1$). Even without regard to the

TABLE 3.—Objective response rate to cytotoxic chemotherapy as a function of estrogen- and progesterone-receptor status

Status	Response rate
Estrogen-receptor positive	
Progesterone-receptor positive	0 (0 of 5 patients)
Progesterone-receptor negative	10% (1 of 10 patients)
Estrogen-receptor negative	
Progesterone-receptor positive	0 (0 of 3 patients)
Progesterone-receptor negative	88% (21 of 24 patients)

TABLE 4.—Objective response rate to cytotoxic chemotherapy as a function of estrogen- and androgen-receptor status

Status	Response rate
Estrogen-receptor positive	
Androgen-receptor positive	0 (0 of 4 patients)
Androgen-receptor negative	0 (0 of 13 patients)
Estrogen-receptor negative	
Androgen-receptor positive	71% (5 of 7 patients)
Androgen-receptor negative	84% (16 of 19 patients)

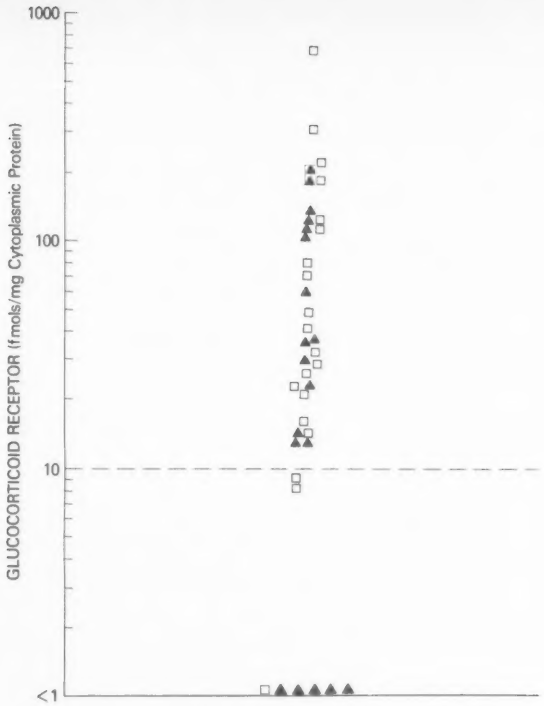

FIGURE 3.—Influence of glucocorticoid-receptor status on objective response rate to cytotoxic chemotherapy in metastatic breast cancer. Objective response is the sum of complete + partial responders.

cutoff value of positivity for the glucocorticoid receptor, the distribution of glucocorticoid-receptor values for responders did not differ significantly by Wilcoxon rank-sum analysis from the corresponding distribution for nonresponders. The response rate to chemotherapy as a function of both the estrogen and glucocorticoid receptors is shown in table 5. The presence or absence of glucocorticoid receptor does not alter the response rate in patients who are either estrogen-receptor positive or negative. No difference between the glucocorticoid-receptor positive and negative groups with regard to age, menopausal status, Karnofsky performance score, disease-free interval, number of sites involved with metastatic disease, or presence of visceral involvement could be identified, which explained why glucocorticoid receptor did not correlate with response to cytotoxic chemotherapy.

DISCUSSION

This report extends our previous work in which we were able to demonstrate an association between estrogen-receptor status and response to cytotoxic

TABLE 5.—Objective response rate to cytotoxic chemotherapy as a function of estrogen- and glucocorticoid-receptor status

Status	Response rate
Estrogen-receptor positive	
Glucocorticoid-receptor positive	0 (0 of 11 patients)
Glucocorticoid-receptor negative	0 (0 of 1 patient)
Estrogen-receptor negative	
Glucocorticoid-receptor positive	70% (14 of 20 patients)
Glucocorticoid-receptor negative	71% (5 of 7 patients)

chemotherapy (2). It was postulated that tumors which lacked the estrogen receptor might be less differentiated than estrogen-receptor positive tumors, have a more rapid growth rate, and thus be more sensitive to chemotherapy. Lack of estrogen receptor and response to chemotherapy were correlated in our retrospective analysis of these 70 patients. We now show that lack of progesterone receptor is also associated with a high response rate to chemotherapy while progesterone-receptor positive tumors have a very low response rate. This differential response rate is not explained by a bias in patient selection criteria. Indeed, the two groups are virtually identical with regard to age, menopausal status, Karnofsky performance score, disease-free interval, presence of visceral disease, number of sites involved with metastatic disease, chemotherapy regimens employed, and amount of prior therapy received. In our estrogen-receptor negative group which had a response rate of 76%, the lack of a progesterone receptor increases this response rate to 88%. The presence of a progesterone receptor has been reported to increase the response rate to endocrine manipulation in patients whose tumors are estrogen-receptor positive (1), and this negative correlation with response to chemotherapy may prove to be a new and useful role for the progesterone receptor. It is generally accepted that in the normal uterus, progesterone receptor is regulated by estrogen (8). A similar relationship has been suggested for human breast cancer (9) and demonstrated for human breast cancer cells in vitro (10). Hormone dependence is generally taken as an indication of tumor differentiation. Thus, one would anticipate that progesterone-receptor loss might well be associated with a less well-differentiated tumor.

The presence or absence of androgen or glucocorticoid receptors did not predict response rate to cytotoxic chemotherapy and did not add to the predictive index of the estrogen receptor. It is known that the administration of pharmacologic doses of androgenic compounds results in tumor regression in approximately 20% of patients with metastatic breast cancer (11). This response rate

has thus far not been correlated with the presence of androgen receptor but is correlated with the presence of estrogen receptors in the tumors (12). Tumor regression occurs in 10%–15% of breast cancer patients receiving glucocorticoids (13), but this response rate has also never been correlated with the presence or absence of glucocorticoid receptor. Glucocorticoid receptors in human acute lymphoblastic leukemia have been shown to predict response to glucocorticoid therapy (14), and in addition seem to correlate strongly with remission induction and duration even in patients receiving cytotoxic chemotherapy (15).

In summary, lack of progesterone receptor in addition to lack of estrogen receptor is associated with an increased response rate to cytotoxic chemotherapy in patients with metastatic breast cancer. No role was identified for either the androgen or glucocorticoid receptors.

REFERENCES

1. McGuire WL, Horwitz KB, Pearson OH, et al. Current status of estrogen and progesterone receptors in breast cancer. Cancer 39:2934–2947, 1977.
2. Lippman ME, Allegra JC, Thompson EB, et al. Lack of estrogen receptor is associated with an increased response rate to cytotoxic chemotherapy in metastatic breast cancer. N Engl J Med 298:1223–1228, 1978.
3. Lippman ME, and Huff K. A demonstration of androgen and estrogen receptors in human breast cancer using a new protamine sulfate assay. Cancer 38:868–874, 1976.
4. Lippman ME, Huff KK, and Bolan G. Progesterone and glucocorticoid interactions with receptors in breast cancer cells in long term culture. Ann NY Acad Sci 286:101–115, 1977.
5. Aitken S, and Lippman ME. A simple computer program for quantification and Scatchard analysis of steroid receptor proteins. J Steroid Biochem 8:77–94, 1977.
6. Breast Cancer Task Force Treatment Committee. Breast Cancer: Suggested Protocol Guidelines for Combination Chemotherapy Trials and for Combined Modality Trials. Bethesda, Md, National Cancer Institute, National Institutes of Health, DHEW Publication No. (NIH) 77–1192.
7. Lehman EH. Non-Parametrics: Statistical Methods Based on Ranks. San Francisco, Holden-Day Inc, 1975, p 208.
8. Toft DO, and O'Malley BW. Target tissue receptors for progesterone: the influence of estrogen treatment. Endocrinology 90:1041–1045, 1972.
9. Horwitz KB, McGuire WL, Pearson OH, et al. Predicting response to endocrine therapy in human breast cancer: a hypothesis. Science 189:726–727, 1975.
10. Horwitz KB, and McGuire WL. Estrogen control of progesterone receptor in human breast cancer: correlation with nuclear processing of estrogen receptor. J Biol Chem. In press.
11. American Medical Association Committee on Research. Androgens and estrogens in the treatment of disseminated mammary carcinoma. JAMA 172:1271, 1960.
12. McGuire WL, Carbone PP, Sears ME, et al. *In* Estrogen Receptors in Human Breast Cancer (McGuire WL, Carbone PP, and Vollmer EP, eds). New York, Raven Press, 1974, pp 1–7.
13. Stoll BA. Androgen, corticosteroid and progestin therapy. *In* Endocrine Therapy in Malignant Disease (Stoll BA, ed). Philadelphia, W. B. Saunders Co, 1972, pp 176–182.
14. Lippman ME, Halterman RH, Leventhal BG, et al. Glucocorticoid binding proteins in acute lymphoblastic leukemic blast cells. J Clin Invest 52:1715–1725, 1973.
15. Konior GS, Lippman ME, Johnson GE, et al. Correlation of glucocorticoid receptor (GR) levels and complete remission duration (CRD) in "poor prognosis" acute lymphatic leukemia (ALL). Proc Am Assoc Cancer Res and ASCO 18:353, 1977.

5-Fluorouracil, Methyl-CCNU, Adriamycin, and Mitomycin C in the Treatment of Advanced Gastric Cancer [1]

Paul A. Bunn, Jr,* John L. Nugent, Daniel C. Ihde, Martin H. Cohen, Joyce L. Eddy, and John D. Minna [2]

SUMMARY

Eighteen previously untreated patients with advanced nonresectable adenocarcinoma of the stomach were treated with a four-drug regimen consisting of 5-fluorouracil, methyl-CCNU, adriamycin, and mitomycin C. Objective responses occurred in only two patients (11%); the majority of patients had stable disease for ≥ 8 weeks (61%). The median survival of 27 weeks was similar to that reported in other combination chemotherapy trials. Numerous staging and restaging procedures and strict response criteria were utilized and each was evaluated for its effectiveness in following responses. None of these parameters was useful for evaluating response of intra-abdominal tumor. We conclude that this four-drug combination is no better than previously described two-drug regimens.

[Cancer Treat Rep 62:1287–1293, 1978]

Adenocarcinoma of the stomach, despite a declining incidence, is still the sixth leading cause of cancer death in the US (1). The median survival time for patients with nonresectable disease who receive no subsequent therapy is only 16 weeks (2). Active chemotherapeutic agents including 5-fluorouracil (5-FU), adriamycin, mitomycin C (MMC), and the nitrosoureas produce objective partial responses in 7%–50% of patients (3–8). Unfortunately, these agents rarely produce complete remissions, and median remission durations are short. Consequently, single-agent chemotherapy has not made an impact on survival. Combination chemotherapy trials combining 5-FU with nitrosoureas (BCNU [9,10], methyl-CCNU [11,12], or streptozotocin [13] or with MMC (14,15) gave higher response rates than did single-agent treatment. However, response durations remained short and median survival was only prolonged to about 27 weeks in the best combination trials (8,9,11).

More recently, three-drug combinations employing 5-FU and MMC with adriamycin or cytosine arabinoside have been reported to produce objective partial responses in as many as 50% of patients, although survival data for these patients have not yet been published (16–19). Because combination chemotherapy appears to increase response rates and may prolong survival, we designed a treatment program combining the four most active chemotherapeutic agents. Since each drug is myelosuppressive and since there are some data in other solid tumors to suggest that cyclic administration of active noncross-resistant combination regimens may improve results over each regimen alone (20,21), we have utilized alternating two-drug combinations given at full dose.

Responses of intra-abdominal tumors are notoriously difficult to evaluate. Consequently, we have employed a number of procedures including plasma carcinoembryonic antigen (CEA), gastroscopy, upper gastrointestinal (UGI) series, peritoneoscopy, and radionuclide scans to evaluate the effectiveness of each parameter in assessing objective tumor response and progression.

METHODS AND MATERIALS

Patient characteristics.—Eighteen previously untreated patients with unresectable histologically

[1]Received Mar 1, 1978; revised June 23, 1978; accepted July 10, 1978.

[2]National Cancer Institute-Veterans' Administration Medical Oncology Branch, Veterans' Administration Hospital, Washington, DC.

Reprint requests to: Paul A. Bunn, Jr, MD, National Cancer Institute-Veterans' Administration Medical Oncology Branch, Veterans' Administration Hospital, 50 Irving St, NW, Washington, DC 20422.

TABLE 1.—Patient characteristics

Patient— No.	Age	Race	Sex	PS*	Sites at diagnosis†	Type of response (No. of cycles of stable disease)	Sites of progression	Survival (wks)
1	61	W	M	1	UGI, endo, bone	Stable (1)	Bone	14
2	57	W	M	1	UGI, endo, bone, liver	Stable (1)	Bone, liver	44
3	49	B	M	1	UGI, endo, liver	Prog‡	Liver, decrease in weight	14
4	27	W	F	1	UGI, endo, abdominal tumor	Partial response	Abdominal tumor, ascites	73
5	53	W	M	2	UGI, endo, skin, lymph nodes, bone, bone marrow	Prog‡	Skin, lymph nodes, ascites, decrease in PS	3
6	60	W	M	2	UGI, endo, liver	Prog‡	Ascites, decrease in PS	9
7	72	W	F	1	UGI, endo, lung	Prog‡	UGI, abdominal tumor, lymph node, decrease in weight, decrease in PS	50
8	64	W	M	2	UGI, endo, abdominal tumor, liver	Partial response	Liver, decrease in weight, decrease in PS	27
9	59	B	F	2	UGI, endo, liver	Stable (1)	Liver, UGI	58+
10	67	W	M	2	UGI, endo	Stable (1)	Pleural effusion, ascites	27
11	57	W	F	1	UGI, endo, abdominal tumor	Stable (1)	Abdominal tumor, liver	38
12	43	W	F	1	UGI, endo	Stable (2)	UGI, lymph node, abdominal tumor, decrease in weight	38
13	48	W	M	1	UGI, endo	Stable (2)	Decrease in weight, decrease in PS	30
14	47	W	M	1	UGI, endo, liver	Stable (1)	Lung, UGI, liver, decrease in weight, decrease in PS	31
15	51	W	M	1	UGI, endo	Stable (1)	Endo,† UGI	27
16	56	B	M	2	UGI, endo, abdominal tumor, liver	Stable (1)	Decrease in weight, decrease in PS, abdominal tumor	14
17	55	B	M	2	UGI, endo, lymph node, lung, liver	Prog‡	UGI, endo,† decrease in weight, lung, liver, decrease in PS	15
18	38	W	F	1	UGI, endo, liver	Stable (2)	-	30+

*Zubrod performance scale: 0 = asymptomatic, fully ambulatory; 1 = symptomatic, fully ambulatory; 2 = symptomatic, ambulatory > 50% of the time; 3 = symptomatic, ambulatory < 50% of the time; 4 = completely bedridden.
†Endo = UGI endoscopy.
‡Prog = progressive disease at 8 wks.

confirmed adenocarcinoma of the stomach were studied between July 1976 and June 1977. Patient characteristics are provided in table 1. Macroscopically, three patients had ulcerating lesions, ten had polypoid or fungating lesions, and five had infiltrating lesions. Histologically, four tumors were well-differentiated and 14 were poorly differentiated. Two patients had previously received palliative partial resections. Both had extensive liver metastases at the time of the study. Patients undergoing palliative resection with residual disease which was non-evaluable (microscopic) were excluded from the study.

Staging procedures.—Pretreatment staging evaluation included complete history and physical examination, complete blood cell and platelet counts, chemistry panel, plasma CEA, bone marrow aspirate and biopsy, liver, spleen, and bone scans, chest roentgenograms, UGI series, and gastroscopy. Patients whose livers were not evaluated at surgery underwent percutaneous liver biopsy which, if negative, was followed by peritoneoscopy with directed biopsies. Gastroscopy, UGI series, and all other positive pretreatment studies were repeated at the conclusion of each 8-week treatment cycle. All patients had evaluable disease on UGI series and

TABLE 2.—Methyl-CCNU, 5-FU, adriamycin, and MMC for advanced gastric carcinoma

Drug*	Drug dose: (mg/m²)/route	Day—													
		1	2	3	4	5	----	29	----	57	58	59	60	61	---- 85
5-FU	325/iv	×	×	×	×	×				×	×	×	×	×	
Methyl-CCNU	150/oral	×								×					
Adriamycin	50/iv							×							×
MMC	10/iv							×							×

* Courses of all 4 drugs repeated at 8-wk intervals.

gastroscopy; four patients had lesions on UGI series and gastroscopy alone (evaluable lesions), two had measurable abdominal tumors, and 12 had measurable metastatic disease (table 1).

Treatment schedule.—Patients were treated with the four-drug regimen consisting of 5-FU, methyl-CCNU, adriamycin, and MMC shown schematically in table 2. Drug doses were modified or delayed for hematologic toxicity as follows: (*a*) if the wbc count was $< 3500/mm^3$ and/or the platelet count was $<100,000/mm^3$ at week 4 or 8 of each cycle, the treatment was delayed for 1 week; (*b*) if at that time the wbc count was 2500–3500/mm³ and/or the platelet count was 75,000–99,000/mm³, the drug doses were decreased by 25%; or (*c*) if the wbc count was $< 2500/mm^3$ and/or the platelet count was $< 75,000/mm^3$, the treatment was delayed until the counts recovered to those levels.

Response criteria.—Complete response was defined as the disappearance of all clinically detectable tumor. A partial response required a decrease in an already measurable lesion of > 50% in the product of the longest perpendicular diameters or an 80% decrease in evaluable lesions as confirmed independently by two observers. Evaluable lesions included abnormalities on UGI series, gastroscopy, roentgenogram, or radionuclide scan which did not have discrete measurable borders. Progressive disease included the occurrence of any new lesion, an increase of > 25% in the product of the longest perpendicular diameters of measurable lesions or > 50% of evaluable lesions, weight loss > 10%, or a decrease in the Zubrod performance status (PS)[3] of > 1 score level in the presence of stable disease. Stable disease was defined as the lack of response or progression for at least 8 weeks.

[3] 0 = asymptomatic, fully ambulatory; 1 = symptomatic, fully ambulatory; 2 = symptomatic, ambulatory > 50% of the time; 3 = symptomatic, ambulatory < 50% of the time; 4 = completely bedridden.

RESULTS

Therapeutic response.—Only two patients (11%) had objective partial responses. Both had measurable lesions and their survival was 73 and 27 weeks. Eleven patients (61%) had stable disease at the completion of one chemotherapy cycle. All 11 patients received a second cycle of therapy; eight progressed during this cycle and three remained stable, but all three of these progressed during a third cycle. Five patients (28%) progressed during the first cycle of therapy. The median survival for the entire group was 27 weeks and actuarial survival is shown in figure 1. The median survival for patients with stable disease after one cycle was 30 weeks (range, 11–50+) and the median survival of patients who progressed during the first cycle was 14 weeks (range, 3–50).

Sites of response and progression (tables 1 and 3).—Since disease response and progression are notoriously difficult to evaluate in gastric carcinoma, UGI series, gastroscopy, chest roentgenograms, bone, liver, and spleen scans, plasma CEA, and physical examination and measurements of weight and PS were performed initially and repeated with each 8-week cycle. Table 1 provides the initial sites of disease, response, sites of progression, and survival for each patient. Table 3 demonstrates the effectiveness of each procedure in determining disease response. Intra-abdominal disease including the stomach and perigastric area but excluding the liver was difficult to evaluate with any parameter. Gastroscopy and UGI series improved in only one of the two responders and worsened in only eight of 14 instances of progressive disease (table 3). In all seven patients in whom it was tried, peritoneoscopy was not useful in evaluating local intra-abdominal disease since adhesions obscured visualization of the stomach. In contrast, metastatic disease was more readily evaluated. Abnormal liver and bone scans became worse in eight of ten instances of disease progression and one initially normal liver scan became abnormal with disease progression.

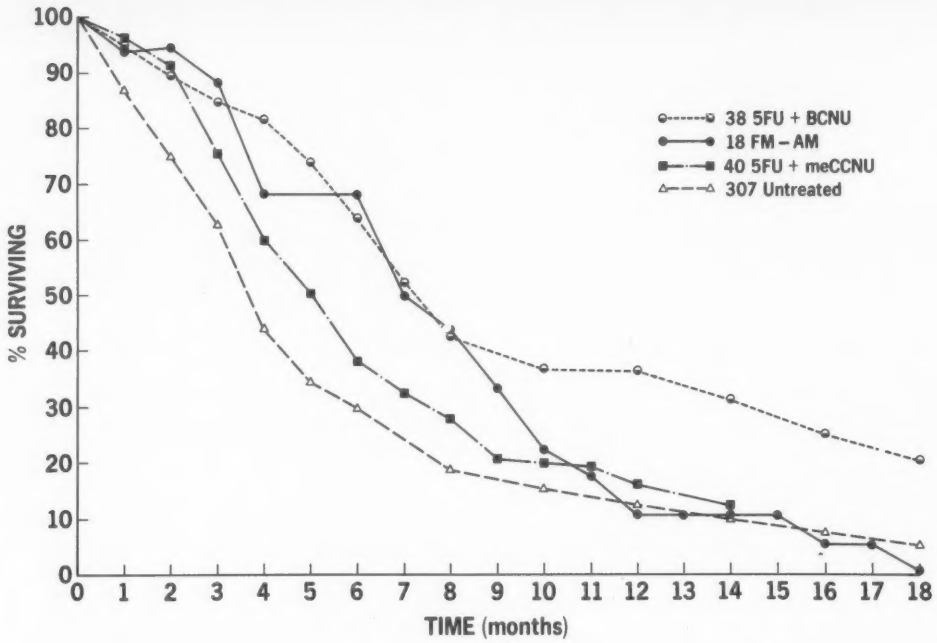

FIGURE 1.—Actuarial survival for 18 5-FU, methyl-CCNU, adriamycin, and MMC (FM-AM) study patients, 38 patients treated with 5-FU and BCNU (9,10), 40 patients treated with 5-FU and methyl-CCNU (12), and 307 untreated patients (2).

TABLE 3.—Efficacy of parameters used to determine response

	All patients	Responding patients	Patients with progression	
Parameter	No. of positive procedures/No. of procedures performed	No. of procedures that improved/No. of procedures performed	No. of procedures that worsened/No. of procedures performed	No. with new lesions/No. of procedures performed
UGI series	18/18	1/2	6/9	—
Gastroscopy	18/18	1/2	2/5	—
Liver scan	9/18	0/1	6/7	1/3
Bone scan	3/18	0/0	2/3	0/3
Chest roentgenogram	2/18	0/0	1/2	1/10
Physical examination	6/18	2/2	4/6	4/11
CEA	11/18	0/2	4/11	0/7
Decrease in weight/ decrease in PS	—	0/2	10/18	—

TABLE 4.—Wbc and platelet count nadirs by cycle

Cycle	Median wbc nadir × 10^3/mm^3 (range)	Median platelet nadir × 10^3/mm^3 (range)	No. of dosage modifications (total No. of patients)
1	2.6 (1.1–10.5)	89 (33–289)	5 (18)
2	2.9 (1.5–7.8)	65 (17–470)	8 (12)
3	1.8 (0.9–2.5)	68 (32–104)	3 (4)

Physical examination was extremely useful; both responders had a total disappearance of palpable abdominal tumors at the time of response. With disease progression, abnormalities that were initially present worsened in four of six patients, and new physical findings developed in four additional patients. Abdominal tumors, malignant ascites, and new or enlarging lymph nodes developed in three patients each. Seven patients underwent peritoneoscopy. In three of these patients, the liver could not be visualized because of adhesions; liver biopsy in one of the remaining four was positive. Serial plasma CEA measurements, performed every 8 weeks in all 18 patients, were not useful in detecting disease response or early disease progression. Initially, plasma CEA was > 5 ng/ml in 11 of 18 patients. Plasma CEA levels did not change significantly in either responder (6→4.6 and 2.1→1.0) and only four patients developed an increase of > 50% with disease progression. Four patients whose CEA had not changed at the time of disease progression developed an increase in CEA 8–16 weeks after disease progression had been documented by other means. CEA never increased in the remaining ten patients despite the development of progressive disease. Weight loss > 10% of the pretreatment weight occurred in eight patients (44%) and PS deteriorated by > 1 Zubrod score level in eight patients with progressive disease. Weight loss and deterioration in PS were the only evidence of progressive disease in one patient who died from intra-abdominal tumor 2 weeks later.

Toxicity (table 4).—All patients developed some nausea and vomiting; this was partially controlled with antiemetics. No patients stopped treatment because of these symptoms. Hematologic toxicity was moderate but progressive. On the first cycle, 39% of the patients developed leukopenia (< 2000 cells/mm^3) and 33% developed thrombocytopenia (< 75,000 platelets/mm^3). Median values for the wbc and platelet counts are provided in table 4. During the second and third cycles, leukopenia and thrombocytopenia were as severe despite frequent modifications in drug dosage (table 4). There were no episodes of congestive heart failure or arrhythmia. There were no severe bleeding episodes, serious infectious complications, or drug-related deaths.

DISCUSSION

Recent studies demonstrated that there are at least four active drugs for the treatment of gastric carcinoma (3–8). Results from combination chemotherapy trials employing these agents are summarized in table 5 and figure 1. These two- and three-drug trials resulted in an average response rate of 34% with a range of 21%–55%. The impact on survival was modest with median survivals of 16 weeks in untreated patients and 20–33 weeks in patients treated with combination chemotherapy (table 5 and fig 1). Our study was designed to administer full doses of four known active chemotherapeutic agents. The objective response rate with this combination (11%) was disappointingly low. The majority of patients (61%) demonstrated no objective change for at least 8 weeks. None of these patients later responded and only three had not progressed by 16 weeks. The median survival of 27 weeks in our patients was poor and reflected the advanced nature of disease; patients with postsurgical residual disease without evaluable lesions were excluded from the study. Nevertheless, survival in our study was similar to survival in other combination chemotherapy trials (fig 1).

The major question is why the response rate in our series is low despite survivals which are similar to those in other trials. Figure 1 and table 5 show there was more variation in response rate than in survival. Overall survival is generally determined by prognostic factors of the patient group and by the effectiveness of the therapy. In this study the prognostic factors, including age, sex, race, PS, and histology, were not different than in other studies (9–19). This suggests that the variations in response rates may be attributed primarily to differences in staging and restaging procedures and in response criteria between studies. In our study, response criteria were strict and numerous restaging procedures were employed. Recent combination chemotherapy trials with 5-FU and methyl-CCNU and rigid response criteria have also given response rates lower than those initially reported in other GI cancer studies (22).

The majority of patients in our study had metastatic disease which was evaluated by physical examination, radionuclide scans, and chest roentgenograms. These procedures were moderately sensitive indicators of disease response and progression. However, adequate procedures for the evaluation of intra-abdominal disease remain elusive. Peritoneoscopy was not useful except for eval-

TABLE 5.—Results of combination chemotherapy trials in gastric carcinoma

Ref No.	Drug regimen	No. of patients	Response rate (%)	Median survival (wks)
9	5-FU + BCNU	34	41	33
11	5-FU + methyl-CCNU	30	40	20
12	5-FU + methyl-CCNU	29	21	~ 20
13	5-FU + streptozotocin	12	33	—
15	5-FU + MMC	15	47	—
18	5-FU + MMC + adriamycin	18	50	~ 32
19	5-FU + MMC + adriamycin	42	24	~ 23
17	5-FU + MMC + cytosine arabinoside	32	31	—
16	5-FU + MMC + cytosine arabinoside	27	55	—
Totals		239	35	20–33
Current study	5-FU + methyl-CCNU + MMC + adriamycin	18	11	27

uation of the liver and adhesions frequently obscured the liver. UGI series and gastroscopy were able to detect only gross changes. Disease progression frequently occurred in other sites at a time when these procedures were unchanged. Plasma CEA was shown to be effective in detecting early relapse after surgery for a variety of GI tumors (23–25). However, later studies demonstrated that it was not reliable in measuring response after chemotherapy (26–28). In this study, plasma CEA was also unreliable as an indicator of disease response and progression.

REFERENCES

1. SILVERBERG E. Cancer statistics, 1977. CA 27:26–41, 1977.
2. MOERTEL CG. The natural history of advanced gastric cancer. Surg Gynecol Obstet 126:1071–1074, 1968.
3. COMIS RL, and CARTER SK. A review of chemotherapy in gastric cancer. Cancer 34:1576–1586, 1974.
4. MOERTEL CG, and REITEMEIER RJ. 5-Fluorouracil therapy of advanced carcinoma of the gastrointestinal tract. Cancer Chemother Rep 25:91–94, 1962.
5. MOERTEL CG, REITEMEIER RJ, and HAHN RG. Mitomycin-C therapy in advanced gastrointestinal cancer. JAMA 204:1045–1048, 1968.
6. DOUGLASS HO, JR, LAVIN PT, and MOERTEL CG. Nitrosoureas; useful agents for the treatment of advanced gastrointestinal cancer. Cancer Treat Rep 60:769–780, 1976.
7. FRYTAK S, MOERTEL CG, SCHUTT AJ, ET AL. Adriamycin (NSC-123127) therapy for advanced gastrointestinal cancer. Cancer Chemother Rep 59:405–409, 1975.
8. CARTER SK, and COMIS RL. Gastric cancer: current status of treatment. J Natl Cancer Inst 58:567–578, 1977.
9. KOVACH JS, MOERTEL CG, SCHUTT AJ, ET AL. A controlled study of combined 1,3-bis-(2-chloroethyl)-1-nitrosourea and 5-fluorouracil therapy for advanced gastric and pancreatic cancer. Cancer 33:563–567, 1974.
10. LOKICH JJ, and SKARIN AT. Combination therapy with 5-fluorouracil (5-FU; NSC-19893) and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU; NSC-409962) for disseminated gastrointestinal carcinoma. Cancer Chemother Rep 56:653–657, 1972.
11. MOERTEL CG, MITTELMAN JA, BAKEMEIER RF, ET AL. Sequential and combination chemotherapy of advanced gastric cancer. Cancer 38:678–682, 1976.
12. BAKER LH, VAITKEVICIUS VK, GEHAN E, ET AL. Randomized prospective trial comparing 5-fluorouracil (NSC-19893) to 5-fluorouracil and methyl-CCNU (NSC-95441) in advanced gastrointestinal cancer. Cancer Treat Rep 60:733–737, 1976.
13. SELIGMAN M, BUKOWSKI RM, GROPPE CW, ET AL. Chemotherapy of metastatic gastrointestinal neoplasms with 5-fluorouracil and streptozotocin. Cancer Treat Rep 61:1375–1377, 1977.
14. REITEMEIER RJ, MOERTEL CG, and HAHN RG. Combination chemotherapy in gastrointestinal cancer. Cancer Res 30:1425–1428, 1970.
15. VAITKEVICIUS VK, BAKER LH, BUROKER TR, ET AL. Chemotherapy of gastrointestinal adenocarcinoma. *In* Cancer Chemotherapy—Fundamental Concepts and Recent Advances. Chicago, Year Book Medical Publishers, Inc, 1975, pp 263–278.
16. OTA K, KURITA S, NISHIMURA M, ET AL. Combination therapy with mitomycin C (NSC-26980), 5-fluorouracil (NSC-19893), and cytosine arabinoside (NSC-63878) for advanced cancer in man. Cancer Chemother Rep 56:373–385, 1972.
17. DE JAGER RL, MAGILL GB, GOLBEY RB, ET AL. Combination chemotherapy with mitomycin C, 5-fluorouracil, and cytosine arabinoside in gastrointestinal cancer. Cancer Treat Rep 60:1373–1375, 1976.
18. MACDONALD J, SCHEIN P, UENO W, ET AL. 5-Fluorouracil (5-FU), mitomycin-C (MMC) and adriamycin (ADR)-FAM: a new combination chemotherapy program for advanced gas-

tric carcinoma. Proc Am Assoc Cancer Res and ASCO 17:264, 1976.

19. FOLMAN R, MAGILL GB, PINSKY C, ET AL. Chemoimmunotherapy with mitomycin, 5-FU, and adriamycin (MIFA) plus *C parvum* in advanced gastric cancer. Proc Am Assoc Cancer Res and ASCO 18:195, 1977.
20. BUNN PA, COHEN MH, IHDE DC, ET AL. Commentary: Advances in small cell bronchogenic carcinoma. Cancer Treat Rep 61:333–342, 1977.
21. CASE DC, JR, YOUNG CW, NISCE L, ET AL. Eight-drug combination chemotherapy (MOPP and ABDV) and local radiotherapy for advanced Hodgkin's disease. Cancer Treat Rep 60:1217–1223, 1976.
22. LOKICH JJ, SKARIN AT, MAYER RJ, ET AL. Lack of effectiveness of combined 5-fluorouracil and methyl-CCNU therapy in advanced colorectal cancer. Cancer 40:2792–2796, 1977.
23. SOROKIN JJ, SUGARBAKER PH, ZAMCHECK N, ET AL. Serial carcinoembryonic antigen assays. Use in detection of cancer recurrence. JAMA 228:49–53, 1974.
24. SUGARBAKER PH, ZAMCHECK N, and MOORE FD. Assessment of serial carcinoembryonic antigen (CEA) assays in postoperative detection of recurrent colorectal cancer. Cancer 38:2310–2315, 1976.
25. MARTIN EW, JR, JAMES KK, HURTUBISE PE, ET AL. The use of CEA as an early indicator for gastrointestinal tumor recurrence and second-look procedures. Cancer 39:440–446, 1977.
26. SKARIN AT, DELWICHE R, ZAMCHECK N, ET AL. Carcinoembryonic antigen: clinical correlation with chemotherapy for metastatic gastrointestinal cancer. Cancer 33:1239–1245, 1974.
27. RAVRY M, MOERTEL CG, SCHUTT AJ, ET AL. Usefulness of serial serum carcinoembryonic antigen (CEA) determinations during anticancer therapy or long-term followup of gastrointestinal carcinoma. Cancer 34:1230–1234, 1974.
28. SHANI A, O'CONNELL MJ, MOERTEL CG, ET AL. Serial plasma carcinoembryonic antigen measurements in the management of metastatic colorectal carcinoma. Ann Intern Med 88:627–630, 1978.

Phase II Clinical Trial With High-Dose Methotrexate Therapy and Citrovorum Factor Rescue [1,2]

W. H. Isacoff,* F. Eilber, H. Tabbarah, P. Klein, M. Dollinger, S. Lemkin, P. Sheehy, L. Cone, B.Rosenbloom, L. Sieger, and J. B. Block[3,4]

SUMMARY

One hundred and thirty-four patients with advanced malignant disease were treated with 496 infusions of high-dose methotrexate (HD-MTX) followed by citrovorum factor rescue. Most patients had failed to respond to previous combination chemotherapy. The overall response rate was 29% with 33 partial responses and six complete responses observed in patients with a variety of tumors. Plasma MTX levels were monitored in all patients during each course of therapy in order to identify those patients with delayed plasma MTX clearance. Patients with abnormally slow rates of plasma MTX decay received escalated doses of citrovorum factor rescue in order to prevent drug-induced toxicity. In general, during this study HD-MTX was well-tolerated. Because serious toxicity was neither frequent, severe, nor unpredictable, its use was not limited. HD-MTX should now be evaluated in well-designed controlled clinical trials to compare its antitumor activity to that of conventional- or standard-dose MTX regimens in diseases where HD therapy appears to have efficacy.

[Cancer Treat Rep 62:1295–1304, 1978]

Antifolate therapy in the treatment of patients with cancer has undergone considerable change within the past 30 years. Initially, aminopterin was used in the treatment of acute lymphoblastic leukemia in small doses approximating 0.1 mg/kg of body weight (1). Subsequently, methotrexate (MTX) was employed weekly or biweekly at doses approaching 1 mg/kg of body weight for a variety of tumors (2,3). Currently, MTX is undergoing clinical evaluation in single doses that may exceed 20 g (4). The experimental basis for these large-dose clinical trials stems from the murine studies by Goldin et al (5). They found that the therapeutic efficacy of MTX was improved in animal models by using large toxic doses in conjunction with an antidote, folinic acid, to selectively rescue normal tissues. For certain malignancies, high-dose (HD) MTX with citrovorum factor rescue (CFR) has been associated with therapeutic responses not normally observed with low- or conventional-dose MTX therapy (6–9). Renewed interest in clinical trials with HD-MTX is in part related to its success in the treatment of metastatic osteogenic sarcoma as reported by Jaffe and Paed (10). More recently, they have noted encouraging results in its preoperative and adjuvant use in the therapy for this disease (11,12).

Although the therapeutic response to HD-MTX may have been enhanced for certain tumors, toxicity and risk to the patient have been, until recently, unacceptable (13–15). As a result, HD-MTX has never been evaluated in a broad phase II clinical

[1]Received Mar 27, 1978; revised June 30, 1978; accepted July 6, 1978.

[2]Supported by Public Health Service grants CA-16042 and CA-21210 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Departments of Medicine and Pediatrics, University of California at Los Angeles-Harbor General Hospital, Torrance; Department of Surgery, University of California at Los Angeles Center for the Health Sciences; Eisenhower Medical Center, Palm Desert, Calif; South Bay Hospital, Redondo Beach, Calif; Torrance Memorial Hospital, Calif; Hoag Memorial Hospital, Newport Beach, Calif; and Brotman Memorial Hospital, Culver City, Calif.

[4]We wish to acknowledge Ann Peale and Marian Dietrich for their technical assistance, Marizon Nimoy for her nursing assistance, and Stella Gonzales for her secretarial assistance in preparing this manuscript.

*_Reprint requests to:_ William H. Isacoff, MD, Division of Medical Oncology, Department of Medicine, University of California at Los Angeles-Harbor General Hospital, 1000 Carson St, Torrance, Calif 90509.

TABLE 1.—HD-MTX therapy in 134 patients with advanced malignant disease

Disease	No. of patients treated	No. of courses of therapy	Average No. of courses/ patient	Total dose of MTX (g)	Average dose/ course (g)
Gastrointestinal cancer					
Colon	22	106	4.8	1147	10.8
Gastric	3	5	1.7	32	6.4
Pancreatic	3	8	2.7	56	7.0
Biliary	1	3	3.0	18	6
Sarcoma					
Osteogenic	8	57	7.1	567	9.9
Rhabdomyosarcoma	5	28	5.6	308	10.9
Leiomyosarcoma	4	12	3	111	9.2
Chondrosarcoma	3	6	2	58	9.7
Giant cell	1	2	2	13	6.5
Neurofibrosarcoma	1	5	5	46	9.1
Liposarcoma	1	7	7	59	8.4
Bronchogenic					
Adenocarcinoma	9	36	4	235	6.5
Squamous cell	5	23	4.6	177	7.7
Large cell	4	18	4.5	132	7.3
Oat cell	2	3	1.5	11	3.6
Malignant melanoma	20	65	3.3	560	8.6
Breast	17	54	3.2	458	8.5
Head and neck	7	18	2.6	100	5.5
Leukemia, acute myelogenous	4	11	2.8	86	7.8
Lymphoma	2	4	2	29	7.3
Thyroid	4	9	2	57	6.3
Renal	3	6	2	46	7.6
Mesothelioma	2	4	2	35	8.7
Medulloblastoma	1	2	2	20	10
Uterine	1	2	2	7	3.5
Vaginal	1	2	2	9	4.5
Totals	134	496	3.7	4376.5	8.8

trial. Furthermore, because of the complexity of most HD protocols, its use has been restricted primarily to University-based or highly specialized cancer centers. Assessments of its antitumor effects have also been limited by the use of a multiplicity of dose regimens, rates of infusion, intervals between courses of therapy, and variations in rescue procedures (4). In addition, there still remains a need for a prospective comparative trial using conventional low-dose MTX in tumors that now seem responsive to HD infusions.

This paper presents the therapeutic observations made on 134 patients who received HD-MTX administered as a 4-hour infusion in doses that ranged between 50 and 300 mg/kg of body weight. All infusions were followed by a standard rescue procedure. The results demonstrate that HD-MTX has a broad spectrum of activity and may be given safely when used under specific clinical circumstances.

Patients

Between July 1975 and August 1977, 134 patients (ages 12–71 years) received 496 courses of HD-MTX therapy followed by CFR (table 1). All patients had advanced, nonresectable, biopsy-proven malignant disease. One hundred and two patients had failed to respond to or had relapsed from standard therapy for their diseases; 32 others received HD-MTX as their initial chemotherapy. All patients had objective parameters of disease which could be measured. There were 29 patients with gastrointestinal malignancies, 23 patients with either soft tissue or osseous sarcomas, 20 patients with bronchogenic carcinoma, 20 patients with malignant melanoma, 17 patients with breast cancer, seven patients with squamous cell carcinoma of the head and neck, and six patients with leukemia or lymphoma. The remaining 12 patients had a variety of other solid

tumors. Patients had Karnofsky performance scores of ≥ 40. All patients were expected to live > 1 month. The patients were treated at one of the following hospitals: University of California at Los Angeles Center for the Health Sciences, Los Angeles County Harbor General Hospital, Torrance Memorial Hospital, South Bay Hospital, Hoag Memorial Hospital, Brotman Memorial Hospital, and the Eisenhower Medical Center. Written consent in accordance with institutional policy was obtained from all patients after being informed as to the investigational nature of the therapy.

Pretreatment Evaluation

Prior to receiving HD-MTX, the following baseline studies were performed: physical examination, measurements of body weight and total body surface area, measurement of all tumors, cbc count with absolute platelet count, urinalysis, serum electrolytes, BUN, serum creatinine, 12-hour creatinine clearance, laboratory tests of liver function, chest roentgenogram, and electrocardiogram. A bone, liver, or brain scan was performed if clinically indicated. Patients were excluded from the study if the BUN was > 20 mg/100 ml, if the serum creatinine was > 1.3 mg/100 ml, or if the creatinine clearance was < 60 ml/minute. No patients were excluded because of leukopenia or thrombocytopenia. During the initial 6 months of the study, patients with malignant effusions were excluded.

Treatment Schedule

All patients were hospitalized for each course of therapy. Iv hydration was begun 12 hours prior to the MTX infusion and was continued for 24 hours. Hydration, consisting of a balanced salt solution in 5% dextrose in water, was administered at a rate of 3 liters/m^2/day. To ensure proper urinary alkalinization, Diamox (500 mg) was given orally on the evening preceding therapy. In addition, some patients received $NaHCO_3$ (44 milliequivalents) given 12 hours before MTX administration in alternate liters of iv fluid. The MTX was given as a 4-hour infusion in 2 liters of fluid at an initial dose of 50–100 mg/kg of body weight. The dose was escalated by 50–100 mg/kg of body weight every 1–3 weeks until the maximum dose of 300 mg/kg was reached. Patients were then maintained on doses ranging from 200 to 300 mg/kg. Vincristine (1.5 mg/m^2 iv, but at a total dose not to exceed 2 mg) was administered midway through the MTX infusion.

Rescue Schedule

CFR was begun 4 hours following the completion of the MTX infusion. When the rate of plasma MTX clearance was adequate, an initial iv dose of 40 mg/m^2 of CF was followed by 11 additional doses of 15 mg at 6-hour intervals. Although plasma MTX clearance is dose-dependent (16), clearance was defined as adequate if plasma MTX concentrations were $< 1 \times 10^{-5}$ M at 24 hours, $< 5 \times 10^{-7}$ M at 48 hours, and $< 5 \times 10^{-8}$ M at 72 hours.

All patients with evidence of delayed plasma MTX clearance received augmented doses of CF along with increased fluid administration. The dose of CF was escalated in the following manner. When the plasma MTX level was $> 1 \times 10^{-5}$ M at 24 hours, 50 mg of CF was given iv every 4 hours until the plasma MTX concentration fell to a value $< 5 \times 10^{-6}$ M. Twenty-four mg of CF was then given every 6 hours until the plasma MTX level was $< 5 \times 10^{-7}$ M, followed by CF (15 mg) every 6 hours until the plasma MTX concentration fell to $< 5 \times 10^{-8}$ M. All patients receiving escalated doses of CF also received iv fluids at a rate of 300 ml/hour.

During each course of therapy, cbc and absolute platelet counts, serum electrolytes, urinalysis, body weight, and total fluid intake and output were monitored daily. Blood samples were drawn 4, 8, 12, 24, and 48 hours after the beginning of the MTX infusion to determine the plasma MTX concentration and serum creatinine levels. MTX concentrations were determined by a competitive protein-binding assay developed by Myers et al (17).

RESULTS

Plasma MTX Levels

Peak plasma MTX concentrations achieved at the end of infusion varied with the dose of MTX administered, and ranged between 1.70×10^{-4} M and 9.30×10^{-4} M. The plasma MTX levels at 24 and 48 hours also varied with the dose (table 2). These data served as the basis for identifying those patients with abnormally slow plasma MTX clearance who would require intensification of the rescue schedule.

Toxicity

Infusions Associated With Delayed Plasma MTX Clearance

In 411 of the 496 infusions (83%), plasma MTX concentrations at 24 and 48 hours fell within an acceptable range as previously described (16). The remaining 85 infusions were associated with plasma

TABLE 2.—Plasma MTX concentration presented as a function of time after infusion of various doses

Dose (mg/kg)	Plasma MTX concentration (expressed in mols/liter) at—		
	4 hrs	24 hrs	48 hrs
50	1.70×10^{-4}	6.76×10^{-7}	1.29×10^{-8}
100	3.72×10^{-4}	8.91×10^{-7}	9.55×10^{-9}
150	6.46×10^{-4}	1.29×10^{-6}	2.63×10^{-8}
200	7.08×10^{-4}	2.46×10^{-6}	3.86×10^{-8}
250	8.18×10^{-4}	5.49×10^{-6}	8.67×10^{-8}
300	9.30×10^{-4}	7.94×10^{-6}	2.02×10^{-7}

levels which were higher than expected for a given dose, placing these patients at appreciable risk for developing significant toxicity. In an attempt to prevent or diminish toxicity when rates of plasma MTX clearance were delayed, the rescue procedure was modified in each patient as described by the protocol above. In most cases, escalation of the CF and fluids was successful in preventing severe toxicity (table 3). There were 12 episodes of significant leukopenia (a wbc count of $< 2000/mm^3$) and nine episodes of significant thrombocytopenia (a platelet count of $< 100,000/mm^3$). The manifestations of myelosuppression were noted within 7–14 days after the MTX infusion. The duration of myelosuppression was short in most cases, ranging from 2 to 6 days. There were 14 infusions associated with significant changes in renal function. Patients were considered to have MTX-induced renal abnormalities if their serum creatinine increased to levels > 50% above the preinfusion value. In 12 of these 14 patients, increases in serum creatinine were transient, with values returning to normal usually within 7 days; myelosuppression occurred in five of these 14 patients.

Among the patients with delayed plasma MTX clearance, all but two were successfully rescued and re-treated without incident. Two patients died of infectious complications following a leukopenic episode which was believed to be drug-induced. In each case, CF was intensified and maintained for periods > 2 weeks. In both patients, the wbc count had returned to normal but death resulted as a complication of infection diagnosed during the period of profound leukopenia (wbc count $< 1000/mm^3$).

Toxicity and Adverse Reactions in Total Patient Population

The toxicity and adverse effects experienced by our patients as a result of the 496 courses of therapy are noted in table 4. Nausea and vomiting was frequent, occurring as a result of 95 infusions. When present, it was usually mild, lasting for periods of 12–24 hours on the day of MTX administration. Diarrhea was mild and was observed in 16 patients; it occurred during the first day of therapy and lasted for periods of 24–48 hours.

TABLE 3.—Toxicity resulting from 85 infusions where plasma MTX clearance was delayed

Toxicity	No. of infusions	% of total infusions
Nausea and/or vomiting	15	18
Mucositis	5	6
Wbc count (cells/mm^3)		
< 1000	6	8
1000–2000	6	7
2000–3000	10	5
Platelet count (cells/mm^3)		
< 50,000	5	6
50,000–100,000	4	4
Renal abnormalities	14	16
Changes in liver enzymes	14	16
Anaphylactoid reactions	3	4
Deaths	2	2

Mucositis was noted as a result of 41 infusions, occurring within 5–7 days after therapy. It was usually mild to moderate and in general lasted for only 48–72 hours. Symptoms were effectively alleviated by oral CF mouthwash and viscous Xylocaine. In seven of these patients, three had tumors involving the oral mucosa, while two received MTX in conjunction with or shortly after a course of radiation therapy to the upper aerodigestive tract.

There were four anaphylactoid reactions which occurred in patients who had received multiple courses of MTX therapy. In each instance, the patient became extremely irritable and complained of severe pruritis shortly after the beginning of the infusion. Within minutes, a urticarial rash was noted primarily over the trunk and extremities. In all cases, the MTX infusion was stopped at the onset of the initial symptom. Patients were then treated with Benadryl and corticosteroids. Three of the four patients had subjective feelings of shortness of breath although on physical examination no wheezing was noted. Fever and chills were associ-

TABLE 4.—Toxicity and adverse effects resulting from 496 HD-MTX infusions

Toxicity and adverse effects	No. of infusions	% of total infusions
Nausea and/or vomiting	95	19
Diarrhea	16	3
Mucositis	41	8
Wbc count (cells/mm³)		
< 1000	10	2
1000–2000	14	3
2000–3000	22	4
Platelet count (cells/mm³)		
< 50,000	15	3
50,000–100,000	7	1
Renal abnormalities	22	4
Elevation of liver enzymes	61	12
Anaphylactoid reactions	4	0.8
Deaths	2	0.4

ated with the above-mentioned symptoms which persisted up to 12 hours following discontinuation of the MTX infusion. All patients received subsequent courses of MTX therapy following pretreatment with Benadryl and corticosteroids just prior to infusion. At the time of re-treatment, there was no fever, chills, or shortness of breath. Two patients did experience recurrence of rash. In all cases where anaphylactoid reactions were noted, measurements of serum creatinine following the interrupted infusions were elevated. In addition, three patients had abnormally high MTX plasma values at 24 hours.

Evidence of toxicity, as demonstrated by laboratory parameters, included abnormal hemograms, changes in renal function, and elevations of liver enzymes. Leukopenia was associated with 46 infusions. The nadir of the leukocyte count occurred on the average by Day 8 with a range of between 4 and 14 days. The median duration of leukopenia was 6 days with a range of between 2 and 21 days, and was defined as the number of days the leukocyte count was less than the preinfusion value. Thrombocytopenia occurred in association with 22 infusions. The duration of thrombocytopenia (defined by the estimated number of days that the absolute platelet count was $< 100{,}000/mm^3$ or below the preinfusion value) was 7 days with a range of between 4 and 21 days. No bleeding problems occurred and no subsequent doses were changed due to thrombocytopenia.

There were 22 infusions associated with abnormalities in renal function. Fourteen infusions were associated with abnormal plasma MTX levels at 24 or 48 hours. All renal abnormalities were noted within the first 72 hours after the completion of the MTX infusion. A return to preinfusion levels occurred in 18 of 22 patients within 2 weeks following therapy.

Changes in liver enzymes were noted following 61 of these 496 infusions. In only two patients were liver enzymes persistently elevated causing a delay in further therapy. In all other patients, the liver enzymes returned to normal within 2 weeks and the patients were re-treated without incidence.

THERAPEUTIC RESULTS

A partial objective response was reported if there was a decrease of $\geq 50\%$ in the product of the largest perpendicular diameters of the most clearly measurable parameters of disease which were chosen prior to therapy. If hepatomegaly was chosen, there must have been a reduction of $\geq 30\%$ in the sum of measurements of the xiphoid process and intracostal margin at the midclavicular line. In addition, there must have been no increase in any other indicator lesion, no new areas of malignant disease, and no significant deterioration in weight, symptoms, or performance status. A complete remission was defined as the total disappearance of all measurable tumor parameters. All patients entered in the study were evaluated for response. A total of 33 partial and six complete responses were observed, for an overall response rate of 29% (table 5). The objective response rates were 14% (gastrointestinal cancer), 35% (sarcomas), 30% (lung cancer), 25% (malignant melanoma), 29% (breast cancer), 43% (squamous cell carcinoma of the head and neck), 67% (leukemia and lymphoma), 50% (thyroid cancer), and 50% (gynecologic malignancies). No objective responses were seen in two patients with mesothelioma, in three with renal cell carcinoma, or in one with medulloblastoma. The response rate in previously untreated patients was 35%, compared to 26% in patients who received previous chemotherapy. There was no obvious factor such as drug dose, drug scheduling, or severity of toxicity which would seem to be predictive of response. Twenty-eight patients received only one course of therapy and 30 patients received two courses of therapy. Only eight objective responses were observed in these 58 patients (14%). Of the remaining 76 patients who received three or more courses, 31 objective responses were observed (41%). In general, responses were evident within 4 weeks after beginning therapy and lasted from periods of 1 to > 24 months.

Once a response was documented, patients were

TABLE 5.—Summary of response to HD-MTX therapy in patients with advanced malignant disease

Disease	No. of patients treated	Response	Duration of response (mos)	Response rate (%)	No. of patients who received previous chemotherapy
Gastrointestinal cancer					
Colon	22	4 partial responses	2, 3, 7, 8	18	20
Gastric	3	None	—	0	3
Pancreatic	3	None	—	0	3
Biliary	1	1 partial response	1	100	1
Sarcoma					
Osteogenic	8	3 partial responses	2, 12, 20	38	4
Rhabdomyosarcoma	5	4 partial responses	4, 4, 8, 12	80	4
Leiomyosarcoma	4	None	—	0	4
Chondrosarcoma	3	None	—	0	3
Giant cell	1	None	—	0	1
Neurofibrosarcoma	1	None	—	0	1
Liposarcoma	1	1 partial response	8	100	1
Bronchogenic					
Adenocarcinoma	9	2 partial responses	2, 11	22	5
Squamous cell	5	1 complete response, 1 partial response	7, 3	40	3
Large cell	4	2 partial responses	3, 12	50	3
Oat cell	2	None	—	0	2
Malignant melanoma	20	3 complete responses, 2 partial responses	2, 4, 14, 11, 2	25	8
Breast	17	1 complete response, 4 partial responses	4, 1, 2, 2, 5	29	17
Head and neck	7	3 partial responses	2, 3, 3	43	2
Leukemia, acute myelogenous	4	2 partial responses	2, 3	50	4
Lymphoma	2	2 partial responses	2, 2	100	2
Thyroid	4	1 complete response, 1 partial response	30, 2	50	4
Renal	3	None	—	0	2
Mesothelioma	2	None	—	0	2
Medulloblastoma	1	None	—	0	1
Uterine	1	None	—	0	1
Vaginal	1	1 partial response	1	100	1
Totals	134	6 complete responses, 33 partial responses		29	102

maintained on HD-MTX therapy until evidence of disease progression. In four responding patients who progressed on maintenance therapy, MTX was subsequently escalated at weekly intervals to doses of 450 mg/kg of body weight. One patient treated for advanced colon cancer achieved a second partial remission. The second response was significant in that it was maintained for 4 months. Three of the eight patients with metastatic osteosarcoma relapsed while receiving HD-MTX at a dose of 200 mg/kg every other week with adriamycin as adjuvant therapy. At the time of relapse, each of three patients received weekly HD-MTX at doses which were escalated to 450 mg/kg of body weight. Two of these patients then achieved a partial response lasting 12 and 20 months respectively.

DISCUSSION

Until recently, MTX, when used in HD schedules, proved to have unacceptable toxicity, thereby limiting its more general use (18–21). Early clinical trials were often associated with high rates of drug-related mortality and morbidity. The clinical manifestations of toxicity in these studies included severe mucositis, acute renal insufficiency, and severe

myelosuppression to the point of being life-threatening in a significant number of patients. Significant toxicity was unpredictable in those studies where it was encountered. Currently, the guidelines for the safe administration of HD-MTX have been defined and established by us and others (13–15,22,23). In general, these predictive guidelines have relied on adequate pretreatment renal function and on the availability of a rapid, reproducible, and reliable assay to monitor plasma MTX levels during therapy. Routine pharmacokinetic monitoring of patients during and immediately following MTX therapy has proved essential for both the rational planning of the rescue schedule and for a better understanding of the mechanisms of toxicity. When delayed plasma MTX clearance is observed, adequate adjustments of CF dose schedules and fluid administration are appropriately made. The rationale to escalate the dose of CF when plasma MTX clearance is delayed has been previously reviewed (13,23–25). The amount of CF required to effectively reverse the cytotoxic effects of MTX increases proportionately with respect to the concentration of plasma MTX. The dose of CF should be large enough to compete with MTX for transport into normal tissue. Effective rescue cannot be expected until the plasma MTX concentration falls below that of CF. If MTX concentrations are elevated and clearance is prolonged after therapy, standard CF doses will not achieve molar concentration of sufficient magnitude to compete effectively with the MTX for entry into the cell and ineffective rescue will result. Our modifications of CFR in this study resulted in satisfactory prevention of toxicity when plasma MTX clearance was delayed. CF administration should be continued until the plasma MTX level falls to $< 1 \times 10^{-8}$ M (26).

Pitman and Frei (15) have monitored serum creatinine concentrations at 24 hours following HD-MTX administration in order to detect which patients would be at risk of developing toxicity because of delayed plasma clearance secondary to diminished renal excretion of drug. A serum creatinine level > 50% above the baseline value was considered significant, requiring increases in oral hydration and a repeat serum creatinine determination at 36 hours; if there was a persistent rise, the dose of CF was increased. In our study, we found the use of serial serum creatinine determinations to be of little value in reliably predicting which patients would be at risk of developing MTX toxicity. Of our 85 infusions where plasma MTX clearance was delayed, 71 were associated with no significant rise in the serum creatinine level during the immediate 72 hours after MTX infusion. Of equal significance was the observation that 22 infusions were associated with increased serum creatinine levels, of which eight had normal plasma MTX clearance where the rescue schedule was not intensified and no toxicity was observed. Among the factors which were associated with nonrenal causes of abnormal plasma MTX clearance were inadequate hydration secondary to severe nausea and vomiting, large tumors, significant peripheral edema, paralytic ileus, malignant effusions, ascites, anaphylactoid reactions, and the binding of MTX to plasma proteins (27,28). The relationship between the presence of circulating complexes of protein and MTX and its toxic or therapeutic effect is as yet unclear.

The presence of malignant effusions is thought to influence toxicity to MTX by acting as a "reservoir" or third space causing prolonged continued release of the drug into the systemic circulation. We have observed that the MTX concentration in these effusions ≥ 24 hours after drug administration is always higher than simultaneously drawn plasma samples; this difference may be as great as tenfold or more. Preliminary studies in our laboratory using dialysis techniques have indicated that this may be due to a high concentration of proteins which bind MTX. It is not yet known whether these proteins are similar to albumin and IgG proteins which have been shown to bind MTX in the plasma in patients being treated on HD protocols (28).

Serious toxicity associated with HD-MTX therapy during this study was infrequent. Of the 496 infusions, 6% were associated with severe mucositis, 5% were associated with a wbc count depression to $< 2000/mm^3$, and 3% were associated with thrombocytopenia to $< 50{,}000$ platelets/mm^3. The drug-related mortality rate was 1.5% (two of 134 patients) of the patients entered and 0.4% of the total courses of MTX administered. The limited toxicity observed with our 4-hour infusion and the fact that the program was generally nonmyelosuppressive suggest that HD-MTX may be given more frequently, even in weekly dose schedules. In addition, it may be easily integrated into combination chemotherapy programs which would incorporate the use of myelosuppressive agents at full doses. The observed morbidity with doses of drug approaching 20 g was neither severe nor sufficiently unpredictable to warrant limitation of the drug's use. However, the lack of such morbidity suggests the potential for modification of the CFR in an effort to further enhance the observed therapeutic effects at a tolerated but potentially more toxic risk to the patient. Currently, we have begun delaying the initiation of CF until 24 hours, with careful monitoring of plasma MTX levels through 72 hours.

In the present study, therapeutic observations

were made after 496 courses of HD-MTX therapy in 134 patients with advanced malignant disease. Most of the patients had failed to respond to previous treatment modalities. The likelihood of response to additional therapy in these previously treated patients should, theoretically, be low because of factors such as progression of disease during prior chemotherapy, a poorer performance status, and a shorter expected median duration of survival when compared to untreated patients. A diminished tolerance to additional chemotherapy might be expected because of irreversible drug-induced toxicity from prior therapy. Our results showed an overall response rate of 29%. The response rate in previously untreated patients was 35%; it was 26% in those patients previously treated with anticancer drugs. Our overall response was encouraging in that MTX was employed as a single agent in a relatively nonmyelosuppressive program. Response rates were broadly distributed by disease. For certain diseases such as carcinoma of the head and neck, advanced osteogenic sarcoma, leukemia and lymphoma, and breast cancer, our results are in agreement with previous clinical experience (10,29–32). However, the high order of response in thyroid cancer, rhabdomyosarcoma, non-oat cell bronchogenic carcinoma, malignant melanoma, and colon cancer was unanticipated.

Of the 15 patients receiving HD-MTX for soft tissue sarcoma, five responses were observed. Four of these five responses were seen in patients with disseminated rhabdomyosarcoma and one response was observed in a patient with advanced liposarcoma. Two of the four patients with rhabdomyosarcoma had pulmonary metastases which responded to HD-MTX in doses ranging between 150 and 200 mg/kg of body weight. Each patient had failed to respond to previous chemotherapy consisting of vincristine, adriamycin, and cyclophosphamide. After a response was observed and maintained for 4 months, these patients had pulmonary resection of residual disease. Surgical resection rendered both patients clinically disease-free. They were then maintained on adjuvant HD-MTX therapy postoperatively. The two other patients with rhabdomyosarcoma had disseminated disease involving the lungs, bone, and soft tissue. Both of the patients had failed to respond to previous chemotherapy consisting of adriamycin alone and in combination with DTIC. Partial responses were observed in both of these patients lasting 8 and 12 months respectively. In contrast to our results, Pratt et al (33) used MTX in various dose schedules to treat nine patients with advanced rhabdomyosarcoma. Three received HD infusions ranging from 100 to 200 mg/kg of body weight. No responses were observed in these three patients. Six other patients received MTX at a dose of 25 mg/m^2 twice weekly. One patient was unevaluable and five patients had no response.

Our patient with liposarcoma had a soft tissue tumor of the right lower extremity with metastases to both lung and bones. He was previously treated with both radiation therapy locally and systemic adriamycin. After his initial course of MTX, a dramatic response was observed and he received maintenance therapy every 3 weeks and remained in partial remission for a period of > 8 months.

Of the 20 patients with malignant melanoma, five objective responses were observed: three complete and two partial. A complete response lasting 14 months was observed in a patient with bony metastases. Four patients with cutaneous or subcutaneous recurrence had responses that were of short duration. No patient with visceral metastasis responded to therapy.

Eighteen patients with non-oat cell bronchogenic carcinoma received HD-MTX therapy. Nine of these patients had adenocarcinoma of the lung; two of these had objective remissions, one lasting for 11 months. Of the five patients with squamous cell carcinoma treated in this study, one obtained a complete remission lasting 7 months and another obtained a partial response lasting 3 months. There were four patients with large cell carcinoma, two of whom experienced a partial remission. Djerassi et al (18) reported objective responses in nine of ten patients with non-oat cell bronchogenic carcinoma after HD-MTX therapy. Two of two patients with adenocarcinoma maintained an objective remission for periods of > 9 months, while seven of eight patients with squamous cell carcinoma had objective improvement. Although our data are not as optimistic as those reported by Djerassi et al, we believe that for non-oat cell bronchogenic carcinoma, HD-MTX did exhibit antitumor activity.

A response rate of 18% was noted in patients with advanced colorectal carcinoma. Two patients had prolonged remissions lasting > 6 months. Bertino (24) noted three objective responses in nine patients treated for colon and gastric carcinomas with HD-MTX that ranged from 0.5 to 1.0 g/m^2 given weekly or biweekly. Vogler and Jacobs (32) compared "mild" HD-MTX given orally to conventional-dose MTX. They reported no advantage in the HD-MTX or in the conventional-dose schedule of therapy. The objective response rate was only 11%. The data in this study demonstrate that HD-MTX was therapeutically beneficial in tumors considered to be refractory to conventional-dose MTX or where the drug had not been previously evalu-

ated. To more adequately evaluate these results, prospectively controlled clinical trials are needed which will compare HD regimens with conventional-dose MTX. The high response rate in this study suggests that such studies are now critically needed.

Specific guidelines are lacking which would define the plasma MTX concentrations necessary to induce a clinical response for a particular malignancy. The concept that there may be a tumor-specific threshold of drug or a plasma concentration threshold for MTX cytotoxicity on tumor cells has been suggested (34).[5] This concept would represent a considerable change in the thinking about the kinetics of MTX drug-induced tumor destruction. If substantiated, there may be a dose dependency for MTX such that appropriate drug concentrations must be achieved within the tumor before responses are expected to be observed. Currently, we are exploring a dose-dependent evaluation of MTX in a prospectively controlled clinical trial to evaluate more fully the spectrum of activity of HD-MTX.

A detailed understanding of the mechanism of action of MTX in human malignant disease is still not available despite the considerable amount of knowledge that has accumulated during the 30 years of its use. The major target of MTX appears to be the inhibition of the enzyme dihydrofolate reductase (DHFR) (35). However, other target sites have been suggested, in particular, thymidylate synthetase (36). DHFR is responsible for converting dihydrofolate to reduced folate co-factors, whose function is that of a carrier of one carbon unit essential in the biosynthesis of the purine ring and in the methylation of deoxyuridylic acid to deoxythymidylic acid, a precursor in DNA synthesis. It is believed that the primary event leading to cell death after exposure to MTX is inhibition of thymidylate synthesis resulting in diminished biosynthesis of DNA. However, synthesis of DNA is significantly impaired only if appreciable concentrations of freely exchangeable unbound MTX accumulate within the cell after complete saturation of DHFR (37,38). Therefore, the endpoint for MTX therapy is not simply complete saturation of all available DHFR, but rather the accumulation of high concentrations of freely exchangeable intracellular drug. Kessel et al (39) have demonstrated that the efficacy of drug penetration into the cell will determine the degree of sensitivity to MTX, with sensitive tumors having increased cellular uptake compared to diminished drug uptake in resistant tumors. MTX resistance may also be related to the tumor's ability to synthesize additional DHFR shortly after MTX exposure in sufficient quantity to replenish the tetrahydrofolate pool, thus leading to resumption of DNA synthesis (40). Recently, Bertino et al (41) have demonstrated that patients who were noted to have leukemic cells resistant to MTX had significant increases in DHFR levels at 24 and 48 hours after MTX exposure compared to the enzyme levels prior to therapy. As plasma levels of MTX decreased, so did the intracellular MTX concentration, indicating a rapid efflux process from the cells. The continued synthesis of DHFR by these cells in the face of decreasing intracellular MTX apparently resulted in sufficient DHFR activity to allow resumption of DNA synthesis as reflected by an increase in the tritiated deoxyuridine incorporation of DNA. It has been concluded from these observations that resistance to MTX is, in part, related to the rapid inducibility of DHFR.

Treatment with MTX should, therefore, be aimed at maintaining plasma levels at molar concentrations of $\geq 10^{-6}$ M for > 36 hours. These high molar concentrations within the systemic circulation should favor a steady state such that the intracellular MTX concentration would be high enough to ensure almost total and irreversible inhibition of DNA synthesis during this 36-hour exposure. The rationale for maintaining high plasma MTX concentrations during and after prolonged infusions of MTX is to increase the net intracellular MTX concentration in order to overcome resistance which is either related to ineffective drug transport or to the rapid induction of new DHFR synthesis. Optimal therapy may, therefore, require frequent exposure to HD-MTX at short intervals between each course, with appropriate dose escalations after repeated drug exposure.

[5]Djerassi I. New rescues for high-dose methotrexate. Presented at the 19th Science Writer's Seminar, American Cancer Society, Sarasota, Fla, Apr 4, 1977.

REFERENCES

1. FARBER S, DIAMOND LK, MERCER RD, ET AL. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid (aminopterin). N Engl J Med 238:787–793, 1948.
2. LIVINGSTON RB, and CARTER SK. Single Agents in Cancer Chemotherapy. New York, IFI/Plenum Press, 1970, pp 130–172.
3. CLINE JB, and HASKELL CM. Cancer Chemotherapy, 2nd ed. Philadelphia, W. B. Saunders Co, 1975, pp 38–41.
4. PENTA JS. Overview of protocols on clinical studies of high-dose methotrexate (NSC-740) with citrovorum factor (NSC-3590) rescue. Cancer Chemother Rep Part 3, vol 6(1):7–12, 1975.
5. GOLDIN A, MANTEL N, GREENHOUSE SW, ET AL. Effect of delayed administration of citrovorum factor on the antileukemic effectiveness of aminopterin in mice. Cancer Res 14:43–48, 1954.

6. GOLDSMITH MA, and CARTER SK. The integration of chemotherapy into a combined modality approach to cancer therapy. V. Squamous cell cancer of the head and neck. Cancer Treat Rev 2:137–158, 1975.
7. DJERASSI I. Methotrexate infusions and intensive supportive care in the management of children with acute lymphocytic leukemia: follow-up report. Cancer Res 27:2561–2564, 1967.
8. BERD D, CORNOG J. DECONTI R, ET AL. Long-term remission in diffuse histiocytic lymphoma treated with combination sequential chemotherapy. Cancer 35:1050–1054, 1975.
9. EILBER FR, and ISACOFF WH. High dose methotrexate therapy for disseminated malignant melanoma. Proc Am Assoc Cancer Res and ASCO 17:262, 1976.
10. JAFFE N, and PAED D. Recent advances in the chemotherapy of metastatic osteogenic sarcoma. Cancer 30:1627–1631, 1972.
11. JAFFE N, FREI E III, TRAGGIS D, ET AL. Adjuvant methotrexate and citrovorum factor treatment of osteogenic sarcoma. N Engl J Med 291:994–997, 1974.
12. JAFFE N, FREI E III, TRAGGIS D, ET AL. Weekly high dose methotrexate-citrovorum factor in osteogenic sarcoma: presurgical treatment of primary tumor and of overt pulmonary metastases. Cancer 39:45–50, 1977.
13. ISACOFF WH, TOWNSEND CM, JR, EILBER FR, ET AL. High-dose methotrexate therapy of solid tumors: observations relating to clinical toxicity. Med Pediatr Oncol 2:319–325, 1976.
14. NIRENBERG A, MOSENDE C, MEHTA BM, ET AL. High-dose methotrexate with citrovorum factor rescue: predictive value of serum methotrexate concentrations and corrective measures to avert toxicity. Cancer Treat Rep 61:779–783, 1977.
15. PITMAN SW, and FREI E, III. Weekly methotrexate-calcium leucovorin rescue: effect of alkalinization on nephrotoxicity; pharmacokinetics in the CNS; and use in CNS non-Hodgkin's lymphoma. Cancer Treat Rep 61:695–701, 1977.
16. ISACOFF WH, MORRISON PF, AROESTY J, ET AL. Pharmacokinetics of high-dose methotrexate with citrovorum factor. Cancer Treat Rep 61:1665–1674, 1977.
17. MYERS CE, LIPPMAN ME, ELIOT HM, ET AL. Competitive protein binding assay for methotrexate. Proc Natl Acad Sci USA 72:3683–3686, 1975.
18. DJERASSI I, ROMINGER CJ, KIM JS, ET AL. Phase I study of high doses of methotrexate with citrovorum factor in patients with lung cancer. Cancer 30:22–29, 1972.
19. LEVITT M, MOSHER MB, DECONTI RC, ET AL. Improved therapeutic index of methotrexate with "leucovorin rescue." Cancer 33:1729–1734, 1973.
20. ROSEN G, SUWANSIRIKUL S, KWON C, ET AL. High-dose methotrexate with citrovorum factor rescue and adriamycin in childhood osteogenic sarcoma. Cancer 33:1151–1163, 1974.
21. VON HOFF DD, PENTA JS, HELMAN LJ, ET AL. Incidence of drug-related deaths secondary to high-dose methotrexate and citrovorum factor administration. Cancer Treat Rep 61:745–748, 1977.
22. BLEYER WA. Methotrexate: clinical pharmacology, current status and therapeutic guidelines. Cancer Treat Rev 4:87–101, 1977.
23. STOLLER RG, HANDE KR, JACOBS SA, ET AL. Use of plasma pharmacokinetics to predict and prevent methotrexate toxicity. N Engl J Med 297:630–633, 1977.
24. BERTINO JR. "Rescue" techniques in cancer chemotherapy: use of leucovorin and other rescue agents after methotrexate treatment. Semin Oncol 4:203–216, 1977.
25. PINEDO HM, ZAHARKO DS, BULL JB, ET AL. The reversal of methotrexate cytotoxicity to mouse bone marrow cell by leucovorin nucleosides. Cancer Res 36:4418–4424, 1976.
26. CHABNER BA, and YOUNG RC. Threshold methotrexate concentration for in vivo inhibition of DNA synthesis in normal and tumorous target tissues. J Clin Invest 52:1804–1811, 1973.
27. ISACOFF WH, and EILBER FR. Toxicity H/D MTX-potential influencing factors and treatment. Proc Am Assoc Cancer Res and ASCO 18:325, 1977.
28. DA COSTA M, ROTHENBERG SP, ISACOFF W, ET AL. IgG and other protein complexes with methotrexate (MTX) in patients receiving high dose therapy. Blood (suppl 1) 50:190, 1977.
29. BERTINO JR, BESTON B, and CAPIZZI R. The role of chemotherapy in the management of cancer of the head and neck: a review. Cancer 36:752–759, 1975.
30. VOGLER WR, and JACOBS J. Toxic and therapeutic effects of methotrexate-folinic acid (leucovorin) in advanced cancer and leukemia. Cancer 28:894–901, 1971.
31. SKARIN AT, ZUCKERMAN KS, PITMAN SW, ET AL. High-dose methotrexate with folinic acid in the treatment of advanced non-Hodgkin lymphoma including CNS involvement. Blood 50:1039–1048, 1977.
32. VOGLER WR, and JACOBS J. High divided dose methotrexate (MTX) with leucovorin (LCV) rescue vs low oral divided dose MTX vs single dose iv MTX in advanced carcinoma of head, neck, breast and colon. Proc Am Assoc Cancer Res and ASCO 16:266, 1975.
33. PRATT CB, ROBERTS D, SHANKS E, ET AL. Response, toxicity, and pharmacokinetics of high-dose methotrexate (NSC-740) with citrovorum factor (NSC-3590) rescue for children with osteosarcoma and other malignant tumors. Cancer Chemother Rep Part 3, vol 6(1):13–18, 1975.
34. BENDER RA. Membrane transport of methotrexate (NSC-740) in human neoplastic cells. Cancer Chemother Rep Part 3, vol 6(1):73–82, 1975.
35. WERKHEISER WC. Specific binding of 4-amino folic analogues by folic acid reductase. J Biol Chem 236:888–893, 1961.
36. BORSA J, and WHITMORE GF. Cell killing studies on the mode of action of methotrexate on L-cells in vitro. Cancer Res 29:737–744, 1969.
37. GOLDMAN ID, LICHTENSTEIN NS, and OLIVERIO VT. Carrier-mediated transport of the folic acid analogue, methotrexate, in the L1210 leukemia cell. J Biol Chem 243:5007–5016, 1968.
38. GOLDMAN ID. The mechanism of action of methotrexate. I. Interaction with a low-affinity intracellular site required for maximum inhibition of deoxyribonucleic acid synthesis in L-cell mouse fibroblasts. Mol Pharmacol 10:257–274, 1974.
39. KESSEL D, HALL TC, and ROBERTS D. Modes of uptake of methotrexate by normal and leukemic human leukocytes in vitro and their relation to drug response. Cancer Res 28:564–570, 1968.
40. HRYNIUK WM, and BERTINO JR. Treatment of leukemia with large doses of methotrexate and folinic acid: clinical-biochemical correlates. J Clin Invest 48:2140–2155, 1969.
41. BERTINO JR, SAWICKI WL, CASHMORE AR, ET AL. Natural resistance to methotrexate in human acute nonlymphocytic leukemia. Cancer Treat Rep 61:667–673, 1977.

Pharmacokinetics of BCNU in Man: A Preliminary Study of 20 Patients [1,2]

Victor A. Levin, William Hoffman, and Robert J. Weinkam [3,4,*]

SUMMARY

Using the technique of direct sample insertion selected ion monitoring chemical ionization mass spectroscopy, BCNU levels were measured in vivo in patients and in vitro in serum, sera ultrafiltrates, and buffered Ringer's solution. The disappearance of BCNU in vitro was found to be first-order with a half-time of 11.6 minutes (± 0.5 SD) in volunteers and 15.6 minutes (± 2.3 SD) in patients. The disappearance from serum was catalyzed by a macromolecular component of the serum and was slowed by serum lipids. Analysis of the pharmacokinetics of BCNU in 20 patients using a two-compartment open model demonstrated a volume of distribution of 3.25 liters/kg (± 1.69 SD), a clearance of 56 ml/minute/kg (± 56 SD), and a transfer constant from the central compartment to the outside (K_{10}) of 0.0324 minute^{-1} (± 41% SD), which was close to the decomposition rate observed for BCNU in serum in vitro. The pharmacokinetics of BCNU in patients may be affected by the percent of body fat and the lipid content of the serum.

[Cancer Treat Rep 62:1305–1312, 1978]

In 1967 DeVita et al (1) reported on the pharmacokinetics of [^{14}C]BCNU in mice, rats, dogs, monkeys, and man, as determined by the modified Bratton-Marshall technique described by Loo and Dion (2). These techniques accurately measured BCNU plasma levels to 1 μg/ml, and at that sensitivity, BCNU levels in human plasma could be monitored for 5–10 minutes after either oral or iv doses. Recently, we developed a chemical ionization mass spectrometric (CIMS) method using [$^{2}H_{8}$]BCNU and selected ion monitoring (SIM) that can quantitate plasma BCNU to 50 ng/ml (3). We report here the use of this technique to measure the in vitro degradation of BCNU in serum and the quantitation of BCNU plasma levels in 20 patients who received the drug as part of their therapy for malignant brain tumors.

MATERIALS AND METHODS

Preparation of deuterated BCNU standards.—[$^{2}H_{8}$]BCNU was obtained from the Drug Development Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md), through a contract with SRI International (Menlo Park, Calif). [$^{2}H_{8}$]BCNU standard solutions were prepared in hexane and stored at –70°C; prior to use, the solutions were removed from the freezer and warmed to room temperature. An exact volume of [$^{2}H_{8}$]BCNU was added to teflon-capped culture tubes using a Hamilton dispenser and syringe. Because [$^{2}H_{8}$]BCNU in hexane decomposes at –70°C at a rate of 2%–3%/week, it was necessary to add known amounts of nondeuterated BCNU (2–3 μg/ml, freshly prepared in hexane) to tubes of standard solution. These were

[1]Received Dec 20, 1977; revised May 12, 1978; accepted June 15, 1978.

[2]Supported in part by Public Health Service grant CA–13525 (National Cancer Institute) and Research Career Development award GM-00007 (R.J. Weinkam) (National Institute of General Medical Sciences) from the National Institutes of Health, Department of Health, Education, and Welfare; the Tim Grutman Memorial Fund; and the American Cancer Society Faculty Research award FRA-155 (V. A. Levin).

[3]Brain Tumor Research Center, Department of Neurological Surgery, School of Medicine, and the Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco.

[4]We would like to thank Drs. David Spence and Daniel Furst who worked with us on the initial patient studies, Martha Roberts, RN, and Elinor Silverstein, RN, for their technical assistance, Herbert Landahl, PhD, for valuable discussions and for the derivation of the formula for CP_{DE}, Nancy Phillips, PhD, for her assistance and advice concerning the skin-fold determination, and Neil Buckley for his editorial assistance.

**Reprint requests to:* Dr. Victor A. Levin, Brain Tumor Research Center, 783 HSW, University of California, San Francisco, Calif 94143.

quantitated by the CIMS technique immediately before the determination of BCNU in serum samples.

Patients.—Patients who participated in the study were on the Chemotherapy Service of our institution. Sera were also obtained from healthy volunteers from among Brain Tumor Research Center personnel. Twenty patients who normally received BCNU chemotherapy as part of their therapy for malignant brain tumors were studied. Prior to the study, SMA-12/60 panel, hematocrit level, wbc, differential, and platelet counts, prothrombin time, radionuclide scintiscan, computed axial tomographic scan, and physical examination were obtained for each patient. All patients volunteered for the study and signed a Human Research Consent form. Unlabeled BCNU was supplied by the Division of Cancer Treatment, National Cancer Institute, and was stored at –70°C. The BCNU solutions were prepared in a final concentration of 5% absolute ethanol in 5% dextrose in water. We found that if the BCNU-ethanol solution was added to 5% dextrose in water to give an ethanol-water concentration of < 4% by volume, BCNU precipitated to the bottom of the iv bag. This gave BCNU blood levels that were at their peak near the beginning of the infusion and decreased as the infusion continued. No precipitation occurred in ethanol-water concentrations > 4% by volume. For these studies, a scalp-vein needle was placed in one arm and a 20-gauge angiocatheter was placed in the opposite arm. BCNU was infused through the scalp-vein needle, and blood samples were obtained from a 20-gauge angiocatheter connected to a three-way stopcock that was kept open with heparinized saline. Blood samples (1 ml) were removed both during the infusion, which lasted for 13–75 minutes, and afterward for up to 28 hours. The blood was immediately placed in preheparinized 2.5-ml polypropylene minicentrifuge tubes and centrifuged at 8000 × *g* for 1 minute in a Brinkman minicentrifuge; within 1 minute, 0.5 ml of plasma was pipetted into 13 × 100-mm teflon-capped culture tubes that contained a standard volume of [$^2H_8$]BCNU in hexane. The tubes were then gently rotated and 4–5 ml of ether was added. After phase separation occurred, the ether layer was pipetted into clean culture tubes which were placed in dry ice until they could be stored at –70°C.

CIMS and SIM techniques.—Mass spectral measurements were made using a Finnegan 3200 Mass Spectrometer operated in the chemical ionization mode. Isobutane at 0.6 torr was used as the reagent gas, with an ion source temperature of 110°C. Mass spectrometer parameters were established to monitor the protonated molecular ions, MH^+ 214 and 216 ([$^{35}Cl_2$]BCNU and [$^{35}Cl^{37}Cl$]BCNU) and 222 and 224 ([$^2H_8{}^{35}Cl_2$]BCNU and [$^2H_8{}^{35}Cl^{37}Cl$]BCNU). The dwell time on each channel was 75 msec, with a total data acquisition time of 40 seconds. For each sample, a vial containing 1 ml of an equivolume mixture of hexane and diethyl ether and a known amount (usually between 1 and 2 μg) of [$^2H_8$]BCNU was prepared from a stock hexane solution of [$^2H_8$]BCNU, stored at –60°C, and calibrated with respect to a weighted sample of BCNU. Plasma samples (usually 0.5 ml) were added to each vial and mixed thoroughly for 20 seconds. The organic layer was pipetted into a conical vial and stored at –70°C until analysis, at which time the hexane/ether was evaporated under nitrogen at 30°C. After extraction with ether, the plasma residue was dissolved in dichloromethane and deposited on the ceramic tip of the direct insertion probe. The probe was inserted to within 3 cm of the source block (110°C), where it was warmed by induction. After sufficient ion current had been generated, as indicated by the oscilloscope signal level, the probe was withdrawn. BCNU plasma concentrations were calculated from the drug-to-internal standard ion current ratios. Details of the analytic methodology and the mass spectrometry data system have been reported (3).

In vitro studies.—The in vitro disappearance of BCNU from the sera of patients and healthy volunteers, ultrafiltrates of serum, heated human serum samples, and phosphate buffered Ringer's solution were measured using the techniques described below. The ultrafiltrate samples, obtained from a patient and a volunteer, were prepared by centrifugation of fresh serum in an Amicon Centriflo (25,000-Dalton exclusion limit) at 1100 × *g* for 45 minutes. All samples were placed in sealed neoprene stoppered vials. The samples were then gassed with 5% CO_2/95% air for 1 minute and the pH was adjusted to 7.4 using approximately 0.2 ml of 0.01 N lactic acid/5 ml of serum. BCNU was dissolved in absolute ethanol to achieve concentrations of approximately 50–150 μg/ml, and 10 μl of ethanol-BCNU solution was added to each vial for every 1 ml of serum, ultrafiltrate, or Ringer's solution. The sealed vials were then agitated in a water bath at 37°C for 4 hours. The sealed vials were gassed with 5% CO_2/95% air at hourly intervals and 0.1-ml samples were removed at varying intervals. BCNU levels were determined using the CIMS technique described above for patient serum samples. At the completion of the studies, the pH was remeasured and found to have varied by < ± 0.05 pH units.

Skin-fold (SF) and body-index (BI) measurements.—SF measurements of triceps, subscapular, peri-umbilical, and suprailiac regions were taken in

TABLE 1.—In vitro half-times for BCNU disappearance at pH 7.4 and 37°C

Media and conditions	No. of patients	Half-time ± SD*
Ringer's solution	2	51.4
Patient sera	10	15.6 ± 2.3†
Normal volunteer sera	6	11.6 ± 0.5†
Ultrafiltrate of normal sera	2	42.3
Preheated normal sera		
56°C × 20 mins	2	17.1
68°C × 20 mins	2	27.0
Normal sera 3 hrs after a fatty meal	4	16.7‡

*All sera and serum ultrafiltrates were studied within 3 hrs after venipuncture.

†$P < 0.01$.

‡$P < 0.01$ when compared to these same 4 volunteers studied prior to eating a fatty meal (half-time = 12.0 ± 0.6). Pooled triglycerides (≈ very low density lipoprotein) were 1.2 mg/ml before eating and 1.7 mg/ml 3 hrs after eating.

triplicate with a Lange caliper using the technique of Durnin and Womersley (4). The average of each area was summed to arrive at the sum of SFs. A BI was computed for each patient using weight to the nearest tenth of a kilogram and height to the nearest tenth of a meter: BI = weight/height^2 = kg/m^2.

Calculations.—Implicit in the interpretation of the data and pharmacokinetic modeling that follows is the assumption that the postinfusion BCNU plasma level (C_{pl}) curves could be characterized by two exponentials, $C_{pl} = Ae^{-\alpha t} + Be^{-\beta t}$. Data were fitted to the exponential expressions by a nonlinear iterative computer program, which was a modification of a program supplied by Dr. Clifford Patlak of the Division of Biometry and Epidemiology, National Institute of Mental Health (Bethesda, Md). The pharmacokinetic model chosen for analyzing patient data was the two-compartment open model shown in figure 1 (5,6). A basic flaw in the model is that BCNU is chemically transformed within cells, resulting in some loss from compartment 2 via degradation; that is, a rate constant k_{20} or k_{23} must exist that is indeterminant.

RESULTS

In Vitro

Table 1 summarizes the findings of the in vitro studies. The in vitro disappearance of BCNU from serum and Ringer's solution over the concentration range of 150–0.1 μg/ml was first-order in all cases.

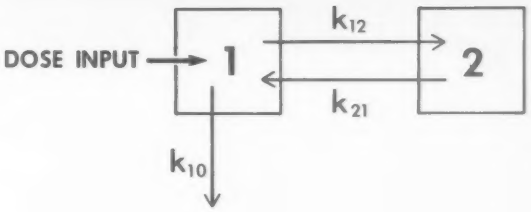

FIGURE 1.—Diagram of the 2-compartment open model (ref 5).

The data best fit the equation $C = Ae^{-\alpha t}$. BCNU degradation in patient and volunteer sera was three times faster than the rate of chemical decomposition observed in buffered Ringer's solution. The factor(s) responsible for this accelerated degradation of BCNU appears to be a macromolecule, since it is filterable and, as the studies on preheated sera demonstrate, is also heat labile. BCNU degradation occurred more rapidly in the sera obtained from healthy volunteers than in the patients' sera.

The lipid content of sera at the time of the study affected BCNU degradation. Sera from the same volunteers were assayed during a period of fasting and 3 hours after a fatty meal. A 41% increase in the half-time of BCNU degradation was found in sera obtained after the meal; this corresponded to a 42% elevation of serum triglycerides from 1.2 to 1.7 mg/ml.

In Vivo

Figure 2 shows four typical patient plasma curves for BCNU disappearance following the infusion period. Plasma levels less than the values shown were below the sensitivity of the assay. Table 2 summarizes the computed pharmacokinetic parameters of 20 patients. CP_{DE} is a dimensionless quantity used to normalize C_0, the peak plasma BCNU level (mg/ml) at the end of infusion, for dose and length of infusion given by

$$CP_{DE} = \frac{C_0}{\text{Dose}} \times \frac{(\text{infusion time})^{2/3}}{37},$$

in which Dose = mg/kg.[5] Although based on infusion plasma drug levels, CP_{DE} should be proportional to the volume of distribution (V_{DSS}).

The other pharmacokinetic parameters were computed assuming a two-compartment open model with primary elimination from the central compart-

[5]Formula derived by Prof. Herbert Landahl, Department of Biophysics and Biomathematics, University of California School of Medicine, San Francisco.

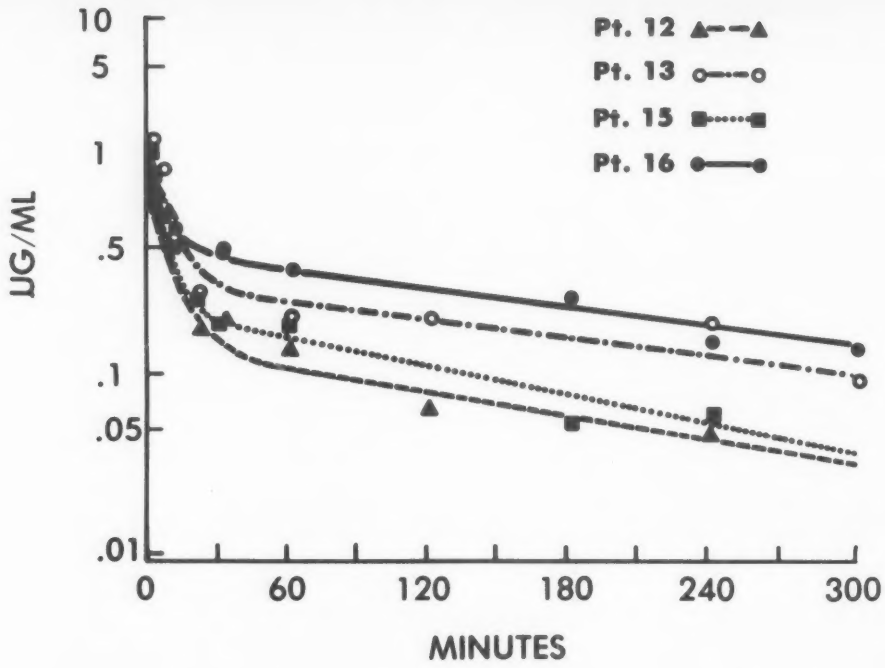

FIGURE 2.—Plasma disappearance curve for BCNU in patient 12, a female who received 70 mg/m² in 40 mins; patient 13, a male who received 80 mg/m² in 32 mins; patient 15, a male who received 70 mg/m² in 33 mins; and patient 16, a female who received 60 mg/m² in 45 mins. Values plotted from end of infusion.

ment. The calculated volume of the central compartment (V_1) was 1.1 liters/kg of body weight, which indicates BCNU distribution into intracellular compartments, whereas the calculated volume of the peripheral compartments (V_2), 2.1 liters/kg of body weight, was almost twice the value for V_1. The half-time for equilibration in the central compartment was 6.1 minutes, and the elimination constant, K_{10}, had a half-time of 21.5 minutes, which is close to that observed for the in vitro degradation of BCNU in serum (15.6 minutes).

SF values were used to determine the percent of body fat from the table of Durnin and Wormsley (4). The mean percent of body fat for our patient population was 31.8 ± 4.0 (SD), which is 1.8-fold greater than found for young males of military age. No doubt this reflects the fact that many of our patients were older and more sedentary and were receiving glucocorticoids. Attempts to correlate SF thickness, BI, or percent of fat with CP_{DE}, V_{Dss} or V_2 using either a single or multiple linear regression analysis were unsuccessful.

To gain some insight into the potential effects of body fat on BCNU distribution, we used Benet's formulas (6) to simulate the amount of BCNU in the central compartment (A_1) and the peripheral compartment (A_2), and the amount degraded and/or eliminated. Figure 3 is a theoretic plot computed from the average values for the 20 patients that appear in table 2. The plot shows that the average patient receiving 2.25 mg/kg of BCNU achieves < 1.2 mg/kg as the maximum amount of drug in the peripheral compartment; by 180 minutes, the peripheral compartment has < 0.3 mg/kg and most of the drug has been degraded.

Figure 4 shows that patient 7, who had the lowest SF value (30 mm), received a dose comparable to the value in figure 3 (2.25 versus 2.35 mg/kg), and the amount eliminated over time is almost the same as for the average of all patients. However, patient 7 had considerably more drug in the central compartment than the peripheral compartment, which is the reverse of the average for all patients.

The dose for patient 9 (fig 5), who had the highest SF value (108 mm), was 38% higher than that for patient 7, which accounts for the higher amount of drug excreted over time as well as the absolute amounts in the central and peripheral compart-

TABLE 2.—BCNU pharmacokinetics in 20 patients: 2-compartment open model (ref 5)*

Patient No.	Dose		Infusion (mins)	SF (mm)	BI (kg/m²)	CP_{DE}	liters/kg			min⁻¹			Clearance (ml/min/kg)
	mg/m²	mg/kg					V_1	V_2	V_{Dss}	K_{10}	K_{12}	K_{21}	
1	160	4.01	48	98.2	22.2	1.29	0.921	1.852	2.773	5.53E-02	4.21E-02	2.10E-02	0.051
2	154	4.30	66	52.3	23.5	1.34	1.094	0.841	1.934	1.69E-02	5.36E-02	6.98E-02	0.018
3	30	0.73	28	74.2	27.4	4.33	0.192	0.507	0.699	2.20E-02	7.08E-02	2.68E-02	0.004
4	60	1.54	35	47.8	22.3	4.17	0.231	0.922	1.153	2.87E-02	1.45E-01	3.64E-02	0.007
5	80	2.00	40	64.5	23.5	1.47	0.714	2.591	3.305	4.82E-03	3.67E-01	1.01E-01	0.003
6	80	2.14	65	43.9	21.4	2.79	0.522	1.259	1.781	3.03E-02	8.93E-02	3.71E-02	0.016
7	80	2.34	35	30.5	19.0	1.24	0.778	0.937	1.715	2.16E-02	3.12E-02	2.59E-02	0.017
8	170	3.38	75	92.6	30.0	0.70	2.289	2.818	5.107	3.66E-02	6.92E-02	5.62E-02	0.084
9	160	3.22	45	108.9	30.6	0.78	1.464	4.045	5.508	3.97E-02	8.39E-02	3.04E-02	0.058
10	80	2.04	31	77.7	23.4	0.36	2.488	1.820	4.307	7.21E-02	2.17E-01	2.97E-01	0.179
11	60	1.55	30	53.7	23.5	0.40	2.189	1.023	3.212	4.57E-02	4.82E-02	1.03E-01	0.100
12	70	1.85	40	72.4	22.5	0.59	1.781	4.712	6.492	3.46E-02	8.66E-02	3.27E-02	0.062
13	80	1.83	32	98.3	25.6	1.12	0.811	3.353	4.164	2.12E-02	9.95E-02	2.40E-02	0.017
14	45	1.31	32	61.1	21.5	0.70	1.306	2.750	4.056	4.95E-02	5.71E-02	2.71E-02	0.065
15	70	1.73	33	74.9	20.2	0.83	1.116	3.819	4.935	3.28E-02	1.03E-01	3.01E-01	0.037
16	60	1.44	45	51.5	25.2	1.03	1.108	1.376	2.484	9.30E-03	7.50E-02	6.04E-02	0.010
17	135	3.09	13.5	66.6	26.1	0.25	2.055	1.527	3.582	6.70E-02	2.24E-01	3.01E-01	0.138
18	80	1.55	15	33.43	31.0	0.90	0.609	4.610	5.219	1.30E-01	3.40E-01	4.49E-02	0.080
19	80	1.49	13	85.8	31.6	0.82	0.606	0.463	1.068	2.98E-01	4.87E-01	6.37E-01	0.180
20	170	3.48	12.5	101	30.1	0.84	0.579	1.001	1.580	5.78E-03	6.65E-01	3.85E-01	0.003
Mean	95	2.25	37	69	25.0	1.30	1.14	2.11	3.25	3.23E-02	1.14E-01	6.29E-02	0.056
SD	45	0.98	17	23	3.9	1.15	0.69	1.38	1.69	41%	36%	44%	0.056
Half-time										21.5 mins	6.1 mins	11.0 mins	

*SF = sum of skin-fold thickness; BI = body index; CP_{DE} (see equation 1); V_1 = volume of central compartment; V_2 = volume of peripheral compartment; $V_{Dss} = V_1 + V_2$; clearance = dose/area under plasma curve.

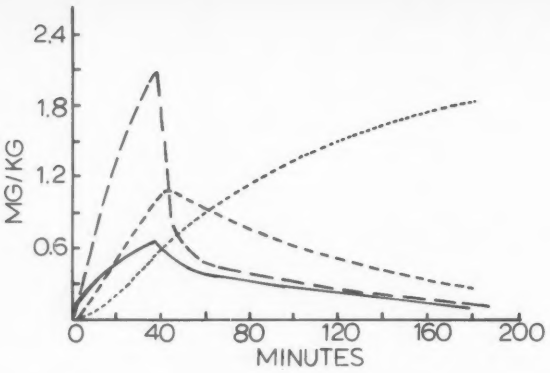

FIGURE 3.—Plot computed by the method of Benet (ref 6) from values averaged for the 20 patients in table 1. ——— = plasma, ——— = amount in A_1, - - - = amount in A_2, and .... = amount degraded and/or eliminated. V_1 = 1.1 liters/kg, V_2 = 2.2 liters/kg, K_{10} = 0.0336 min^{-1}, K_{12} = 0.1427 min^{-1}, K_{21} = 0.0696 min^{-1}, and clearance = 36 ml/kg.

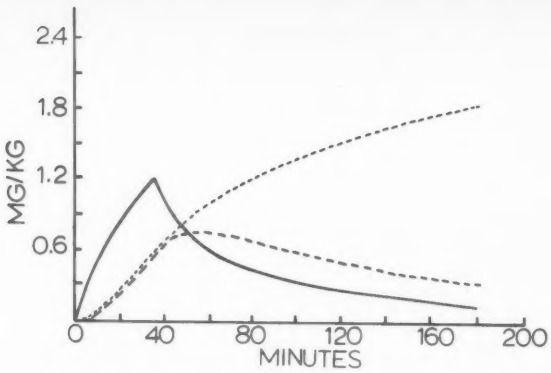

FIGURE 4.—Plot computed by the method of Benet (ref 6) for patient 7 with the lowest SF value (30 mm). ——— = amount in compartment A_1, - - - = amount in compartment A_2, and .... = amount degraded and/or eliminated.

ments. It does not explain the twofold increase in the amount of drug in the central compartment and the 60% reduction in the amount of drug in the peripheral compartment of patient 9 as compared with patient 7. The preponderance of adipose tissue in the peripheral compartment could account for this finding. This correlation, however, holds only at the SF extremes of this study, and could not be corroborated by an in-depth analysis of the remaining 18 patients.

DISCUSSION

The pharmacokinetic studies of BCNU reported here demonstrate the feasibility of using SIM chemical ionization mass spectroscopy to obtain a reliable and reproducible quantitation of BCNU degradation in a variety of aqueous media and in the serum of patients receiving the drug.

The in vitro studies demonstrate the existence of a macromolecule, > 25,000 Daltons, that is heat labile and accelerates the disappearance of BCNU. The BCNU-specific glutathione-*s*-transferase (E.C.2.5.1.18) discovered by Hill in the liver and kidney of mouse and rat (7) was eliminated as the specific macromolecule after Hill failed to identify a similar enzyme in human serum.[6]

Our studies of the in vitro disappearance of BCNU from serum show (table 1) differences between volunteer sera and pretreated patient sera, the latter being 32% higher ($P < 0.01$). While this variation may be partially explained by differences in the catalytic activity of the macromolecular factor, it is also possible that it is affected by the lipid content of the plasma at the time of study. This possibility was supported by our observation that the half-time for BCNU degradation increased 41% in the sera of normal volunteers who had eaten a meal of high lipid content, compared to the rate of degradation observed before the meal was eaten. This observation is significant since patients eat varying amounts of food before they are infused with BCNU, and many others receive glucocorticoids, which can also elevate plasma lipid levels, as part of their therapy. Therefore, considerable patient-to-patient fluctuations in serum lipid levels are expected, and varying BCNU degradation rates would be anticipated as a result.

Studies are underway to determine to what extent specific plasma lipid fractions are responsible for stabilizing BCNU and slowing its degradation in vivo.

Thus, two factors can possibly affect serum breakdown of BCNU: a macromolecule (eg, albumin) accelerates BCNU degradation, and triglycerides (very low-density lipoproteins) may decrease the rate of BCNU degradation in serum. The fact that the half-time for the elimination constant K_{10} (21.5 minutes) is similar to the half-time for the in vitro disappearance of BCNU from patient serum (15.6 minutes) implies a strong influence of serum factors on in vivo pharmacokinetics. Two other factors influencing the in vivo pharmacokinetics of a highly lipophilic drug such as BCNU (log P = 1.5) are body

[6]Hill DL. Personal communication, 1977.

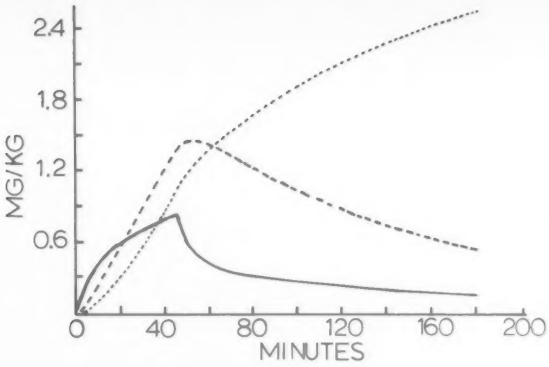

FIGURE 5.—Plot computed by the method of Benet (ref 6) for patient 9 with the highest SF value (108 mm). ——— = amount in compartment A_1, – – – = amount in compartment A_2, and .... = amount degraded and/or eliminated.

TABLE 3.—Tissue masses and tissue/plasma partition ratios used in computation of V_D

Tissue	Weight* (kg)	Partition ratio†
Brain	1.5	0.6
Fat	12.2	5.6
Liver	1.5	0.2
Muscle	33.0	1.4
Kidney	0.3	10.0
Lung	0.6	1.3
Blood	5.4	1.0
Miscellaneous		
Rapidly equilibrated	0.6	5.6‡
Slowly equilibrated	12.6	1.3‡

*From Benowitz et al (ref 11).
†From Levin et al (ref 10).
‡Value approximated from definition given by Benowitz et al (ref 11) for these compartments.

fat content and changes in the pattern of blood flow to body organs (9,10).

Since the volume of distribution (V_{Dss}) of BCNU in the whole body should be a function of the sum of the partition ratios of the drug in tissue compared to plasma, we hypothesized that if partition ratios in man were similar to those previously determined in rat, and if we used the weight of organs given by Benowitz et al (11) for man, we might be able to approximate a volume of distribution for BCNU in the human body independent of the pharmacokinetic parameters for each patient but specific nonetheless for the patient as a function of percent of body fat. Using the constants given in table 3, a volume of distribution (V_D) could be computed using the formula

$$V_D = (\text{fat ratio} \times 5.6) + ([1 - \text{fat ratio}] \times 1.4),$$

in which 5.6 is the partition ratio for BCNU in fat and 1.4 is the mean partition ratio for BCNU in the remaining body compartments. The value of V_D so computed, 2.7 ± 0.2 (SD) liters/kg, while lower than the V_{Dss} of 3.25 ± 1.69 liters/kg computed from the pharmacokinetic values, is nonetheless a reasonable approximation of V_{Dss}.

Because BCNU degrades in vivo at a relatively fast rate, it is likely that small variations in body fat might not produce a consistent measurable effect in BCNU pharmacokinetics and body distribution in a "normal" patient population. Alternatively, BCNU degradation might be so sensitive to minor variations in numerous factors that any significant difference in pharmacokinetics for any isolated factor would be masked by the sum of the action of all factors. In any case, it seems unlikely that any correlation between BCNU pharmacokinetics and body fat can be made except at extreme limits.

This study indicates that BCNU decomposition in human serum in vitro can be catalyzed by a heat-labile macromolecule(s) and can be stabilized by serum lipids. Since the in vivo elimination constant for BCNU approximates the constant for in vitro elimination, factors that affect the latter may have a profound influence on the in vivo pharmacokinetics of BCNU. Finally, even though we were not able to demonstrate a precise relationship between body fat content and BCNU pharmacokinetics, it is likely that percent of body fat does play a role in the pharmacokinetics of BCNU and, in a subtle way, might be expected to have an effect on the delivery of drug to the target organ and tumor, as well as to the bone marrow.

We are now performing detailed studies of the macromolecular and lipid factors that affect the catalysis of BCNU in an effort to explain the variations in the plasma pharmacokinetic parameters observed in this study. These studies will provide information that is important to our improved clinical applications of BCNU as a therapeutic anticancer agent.

REFERENCES

1. DEVITA VT, DENHAM C, DAVIDSON JD, ET AL. The physiological disposition of the carcinostatic 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in man and animals. Clin Pharmacol Ther 8:566–577, 1967.
2. LOO TL, and DION RL. Colorimetric method for the deter-

mination of 1,3-bis(2-chloroethyl)-1-nitrosourea. J Pharm Sci 54:809–810, 1965.

3. WEINKAM RJ, WEN JHC, FURST DE, ET AL. Analysis for 1,3-bis-(2-chloroethyl)-1-nitrosourea using chemical ionization mass spectrometry. Clin Chem 24:45–49, 1978.
4. DURNIN JVGA, and WOMERSLEY J. Body fat assessed from total body density and its estimation from skinfold thickness: measurement on 481 men and women aged from 16–72 years. Br J Nutr 32:77–96, 1974.
5. BENET LZ, and RONFELD RA. Volume terms in pharmacokinetics. J Pharm Sci 58:639–641, 1969.
6. BENET LZ. General treatment of linear mammillary models with elimination from any compartment as used in pharmacokinetics. J Pharm Sci 61:536–541, 1972.
7. HILL DL. *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU), a substrate for glutathione (GSH) *S*-transferase. Proc Am Assoc Cancer Res and ASCO 17:52, 1976.
8. HILL DL, KIRK MC, and STRUCK RF. Microsomal metabolism of nitrosoureas. Cancer Res 35:296–301, 1975.
9. LEVIN VA, LANDAHL H, and FREEMAN-DOVE MA. The application of brain capillary permeability coefficient measurements to pathologic conditions and the selection of agents which cross the blood-brain barrier. J Pharmacokinet Biopharm 4:499–519, 1976.
10. LEVIN VA, KABRA PM, and FREEMAN-DOVE MA. Relationship of BCNU and CCNU pharmacokinetics to uptake, distribution, and tissue/plasma partitioning in rat organs and intracerebral tumors. Cancer Chemother Pharmacol. In press.
11. BENOWITZ N, FORSYTH RP, MELMON KL, ET AL. Lidocaine disposition kinetics in monkey and man. II. Effects of hemorrhage and sympathomimetic drug administration. Clin Pharmacol Ther 16:99–109, 1974.

Kinetics of Cytotoxicity of VM-26 and VP-16-213 on L1210 Leukemia and Hematopoietic Stem Cells [1,2]

Teresa J. Vietti, Frederick A. Valeriote,* Randy Kalish, and Dean Coulter [3]

SUMMARY

The in vivo effects of VM-26 and VP-16-213 upon hematopoietic stem cells and L1210 leukemia cells were quantitated using the spleen-colony assay technique. The effect of VM-26 on leukemia cells was further quantitated using an endpoint dilution assay. The dose-response curves for leukemic clonogenic cells for both agents when given by iv injection were exponential and biphasic, indicating the existence of two populations. The survival of leukemia cells when VM-26 was administered as a 24-hour infusion was less than that found for equivalent doses administered as single injections. The survival kinetics with respect to time for a low dose (0.02 mg/mouse) and a high dose (0.15 mg/mouse) of VM-26 were similar in that the decrease in survival was rapid, reaching a maximum effect within 4 hours after administration. With the low dose, repopulation of the femoral marrow started immediately, whereas with the high dose, there was a 20-hour delay in repopulation. The data from the increase in lifespan studies were in excellent agreement with the quantitative assays. The dose-response curves for the normal hematopoietic clonogenic cells were also exponential, but these cells were much less sensitive to the agents than were the leukemia cells.

[Cancer Treat Rep 62:1313–1320, 1978]

VM-26 and VP-16-213 are semisynthetic derivatives of podophyllotoxin (1). Both compounds decrease the mitotic index of treated cells by arresting cells in late S or G_2 phase of the cell cycle (2–4), although higher concentrations can arrest cells in the S phase as well (4,5). Unlike other podophyllotoxin analogs, metaphase arrest is not seen with VM-26 or VP-16-213 (6,7), and in contrast to podophyllotoxin, VP-16-213 does not inhibit microtubule assembly (7). Also, unlike podophyllotoxin, VP-16-213 and VM-26 cause single-stranded DNA breaks in mammalian cells (8); although this damage can be repaired, it may be responsible for both the cytotoxicity and observable chromosomal aberrations (9). These aberrations are of the chromatid type, suggesting that the major locus of action of VP-16-213 is in G_2. Age-response data indicate that VP-16-213 is most cytotoxic to cells in S and G_2 phases, although it kills cells in all phases of the cycle (4). Thymidine and uridine uptake and their incorporation into macromolecules are inhibited by both agents, although the concentrations required for these effects are orders of magnitude higher than those required for inhibition of cell proliferation (2–9). The finding that DNA, RNA, and protein content per cell (in mass cultures) continues to increase in cells exposed to doses which completely inhibit proliferation is expected if the drug does not block the passage of cells through S phase (2). Additionally, VM-26 inhibits electron transport at the NADH dehydrogenase level (10) and this interference with energy metabolism may be directly responsible for some of the other effects observed.

Both drugs demonstrate significant cytotoxicity in vitro (2,6,11) and to a variety of solid tumors and

[1]Received Jan 4, 1978; revised June 26, 1978; accepted July 11, 1978.

[2]Supported by Public Health Service grant CA-13053 and contract N01-CM-43731 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Division of Hematology and Oncology (*T. J. Vietti, R. Kalish,* and *D. Coulter*) and Division of Radiation Oncology (*F. A. Valeriote*), Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Mo.

**Reprint requests to:* Frederick A. Valeriote, PhD, Section of Cancer Biology, Division of Radiation Oncology, Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Mo 63110.

leukemias in vivo (6,12–14). Both agents show a similar spectrum of response with activity against Hodgkin's disease, reticulum cell sarcoma, lymphosarcoma, and various solid tumors (13,15–17). Each agent has shown encouraging results against specific tumor types. VM-26, for example, shows potential against brain tumors (11,18) and Hodgkin's disease (19), while VP-16-213 has been effective against acute monocytic leukemia (13,15). Because of these results and demonstrated effectiveness of combination chemotherapy in an experimental system (20), it is likely that extensive testing of these agents will continue.

The studies reported here describe the kinetics of action of both VM-26 and VP-16-213 and compare the in vivo effects of both drugs upon hematopoietic stem cells and L1210 leukemia as assayed by the spleen-colony technique.

MATERIALS AND METHODS

Mice.—$CD2F_1$ mice (female BALB/c × male DBA/2), 6–8 weeks old and weighing 20–24 g, were obtained from the National Cancer Institute, Bethesda, Md, for use in these experiments. The mice were kept in our colony for 2 weeks before use and were given food and water ad libitum.

Leukemia cells.—The L1210 line has been previously described (21). It is passaged weekly in male DBA/2 mice. Cells for both the passage and the experiments were obtained from the spleens of DBA/2 mice that received 10^4 cells iv 7 days previously. The spleens were minced through a stainless steel screen (80 mesh) using 10 ml of α-MEM (Flow Laboratories, Inc, Rockville, Md) per spleen. Cell counts of the resultant suspension were performed with a hemocytometer; the suspension was then diluted with α-MEM. For the dose- and time-survival studies, we used $CD2F_1$ mice that had received 2×10^6 cells iv in 0.5 ml via the tail vein 4 days previously. Drugs were injected iv in all experiments. For infusion studies, the drugs were given via a butterfly infusion set attached to a syringe on a Harvard infusion pump (Harvard Apparatus Co, Inc, Millis, Mass).

Spleen-colony assay for leukemic colony-forming units (LCFU).—The use of the spleen-colony assay for L1210 cells has been described (22). Cell suspensions obtained from the femoral marrow of four donor mice were diluted so that 0.5 ml of the final dilution produced two to four macroscopic colonies on the spleen of each of eight recipient mice 8 days later. The number of colonies is proportional to the number of LCFU present in the original marrow suspensions (23). The results were normalized to an untreated control and expressed in terms of fractional survival.

Spleen-colony assay for normal colony-forming units (NCFU).—NCFU were assayed in the femoral marrow of mice by a method previously described (21). Dilutions of this marrow from groups of four normal donor mice were injected iv into groups of ten supralethally irradiated recipients. The colonies were counted 10 days later. As with the LCFU, results are expressed as fractional survival of an untreated control.

Endpoint dilution assay for malignant cells.—To assay for malignant cells, we employed a technique initially proposed by Hewitt (24). Spleens of four leukemic mice were removed and minced through a stainless steel screen with 20 ml of α-MEM. Tenfold dilutions of the resultant suspension were made and, for given dilutions, 1 ml was injected ip into each of six recipient mice. Cell concentrations were adjusted so that all of the mice receiving the highest concentration died whereas none of those receiving the lowest concentration died. Survival of the recipients was followed for 30 days. We calculated the number of malignant cells in the initial suspension using the method described by Fisher and Yates (25).

Increase in lifespan (ILS) studies.—Groups of ten male $CD2F_1$ mice that had received 10^5 leukemia cells iv 2 days previously were treated with different doses of VM-26; their survival was followed for 30 days. The median survival time (MST) was calculated and the ILS was determined as the absolute increase in survival time (to the nearest $^1/_2$ day) compared to an untreated control group. The control groups had an average MST of 8 days.

Drugs.—VM-26 and VP-16-213 were obtained in 5-ml ampules from the National Cancer Institute. Each 50-mg ampule of VM-26 contained solvent composed of *N,N*-dimethylacetamide, polyethoxylated castor oil, maleic acid, benzylol, and ethanol. Each 100-mg ampule of VP-16-213 contained solvent composed of purified polysorbate 80, polyethylene glycol 300, anhydrous citric acid, benzylol, and ethanol. Both solutions were further diluted with normal saline and were administered as either a 0.5-ml bolus injection in the tail vein or a 2.5-ml 24-hour infusion.

Toxicity studies.—Groups of five or ten male $CD2F_1$ mice were given varying doses of VM-26 either by iv bolus injection or by 24-hour infusion and their survival was scored for 30 days. The results were analyzed by probit analysis to determine the LD10, LD50, and LD90 of the agents.

RESULTS

Dose-Survival of LCFU and NCFU by Iv Bolus Injection

Both normal and leukemic mice were given increasing doses of VM-26 or VP-16-213 administered by iv bolus injection; 24 hours later their femoral marrows or spleens were assayed for their CFU content of normal or of malignant cells respectively. Results were compared to an untreated control group assayed at the same time. Data were fit by least-squares analysis over the intervals indicated. For the effect of VM-26 on LCFU, data from both the spleen-colony and endpoint dilution assays are shown together (fig 1). By using the latter assay, we were able to reach survival fractions in the order of 10^{-7}. The VM-26 dose-survival curve was exponential and biphasic; the $D_{\frac{1}{2}}$ (the dose which produces a surviving fraction of 50% on the exponential portion of the curve) was 0.006 mg/mouse for doses < 0.05 mg/mouse and 0.08 mg/mouse for doses between 0.15 and 1.0 mg/mouse. The dose-response curve also appeared biphasic for NCFU over the dose range examined. For doses up to 0.8 mg/mouse, the $D_{\frac{1}{2}}$ was 0.17 mg/mouse and for higher doses, the $D_{\frac{1}{2}}$ was 0.05 mg/mouse.

The dose-survival curve for VP-16-213 (fig 2) was exponential and also appeared to be multiphasic, with a $D_{\frac{1}{2}}$ of 0.04 mg/mouse for doses between 0.1 and 0.5 mg/mouse and a $D_{\frac{1}{2}}$ of 0.12 mg/mouse for doses between 0.5 and 1 mg/mouse. The dose-survival curve for NCFU was exponential with a $D_{\frac{1}{2}}$ of 1.2 mg/mouse. One criterion for measuring a drug's efficacy is its differential cytotoxicity against tumor cells compared to normal cells in a given experimental system. In the past, we have used the ratio of the $D_{\frac{1}{2}}$ values found for the leukemia and normal CFU. Considering only the first portion of the dose-survival curves described above, this ratio is 28 for VM-26 and 30 for VP-16-213.

Tumor-bearing mice were treated with the VM-26 vehicle alone (0.1 and 0.5 ml/mouse), and in two separate experiments LCFU survival was about 3×10^{-1} for both doses.

Infusion, a common procedure for administering many other anticancer agents, has also been recommended for these agents to decrease the incidence of hypotension and to minimize solvent toxicity. We examined the effect of increasing doses of VM-26 given as a 24-hour infusion on both animal survival in normal mice (*see* below) and LCFU survival in leukemic mice. The results for the latter are shown in figure 3 and are compared to the data obtained for bolus injection. No difference was observed in the cytotoxicity between these two schedules, although the infusion schedule may produce greater cytotoxicity at higher doses.

Time-Survival of LCFU

Leukemic mice were given either 0.02 or 0.15 mg of VM-26 and from 15 minutes to 96 hours later their femoral marrow was assayed for LCFU. Results were compared to an untreated control group assayed at 0 hour and are shown in figure 4. For both doses, the survival kinetics were similar. There was an immediate dose-dependent cell-killing with survival reaching a minimum at about 4 hours; repopulation began at the control rate after a time interval dependent upon the dose. For 0.02 mg/mouse, fractional survival decreased to 3×10^{-1} within 15 minutes and then decreased over the next 4 hours to about 5×10^{-2}. Repopulation began at about 8 hours. Following 0.15 mg/mouse, there was a rapid decrease in survival to 2×10^{-3} within 15 minutes with a further decrease to 10^{-4} by 4 hours. Survival remained at this level until about 16 hours, followed by repopulation of the marrow.

ILS Studies

In order to compare our LCFU findings to the results of other investigators and to ascertain the relationship between the drug response at the cellular and whole-animal levels, we carried out ILS determinations in leukemic mice treated with VM-26. The results of five separate experiments are shown in figure 5. Survival increased with increasing doses of VM-26 in a biphasic manner. There was a rapid increase in MST to 0.1 mg/mouse followed by a less rapid increase to 0.4 mg/mouse where the ILS was about 7 days above control. For the 0.6-mg/mouse dose the results were quite variable, with some groups showing a high cure rate and others showing no increase in survival. This indicates that this dose is sufficient to eradicate nearly all leukemia cells in the host, but is close to the toxic level. For doses > 0.6 mg/mouse, the ILS decreased rapidly, reaching a negative value for doses of ≥ 0.7 mg/mouse, indicative of host toxicity.

In order to directly compare the LCFU results with these ILS results, we first obtained from figure 1 the LCFU survival values for the doses used in the ILS studies. We assumed that there was no lag in LCFU proliferation following treatment and that the cells grew with the control doubling time of 9 hours. From this we calculated the expected increase in MST (dashed curve in fig 5). Thus, for doses below the toxic level, good agreement is obtained between the results predicted by the clon-

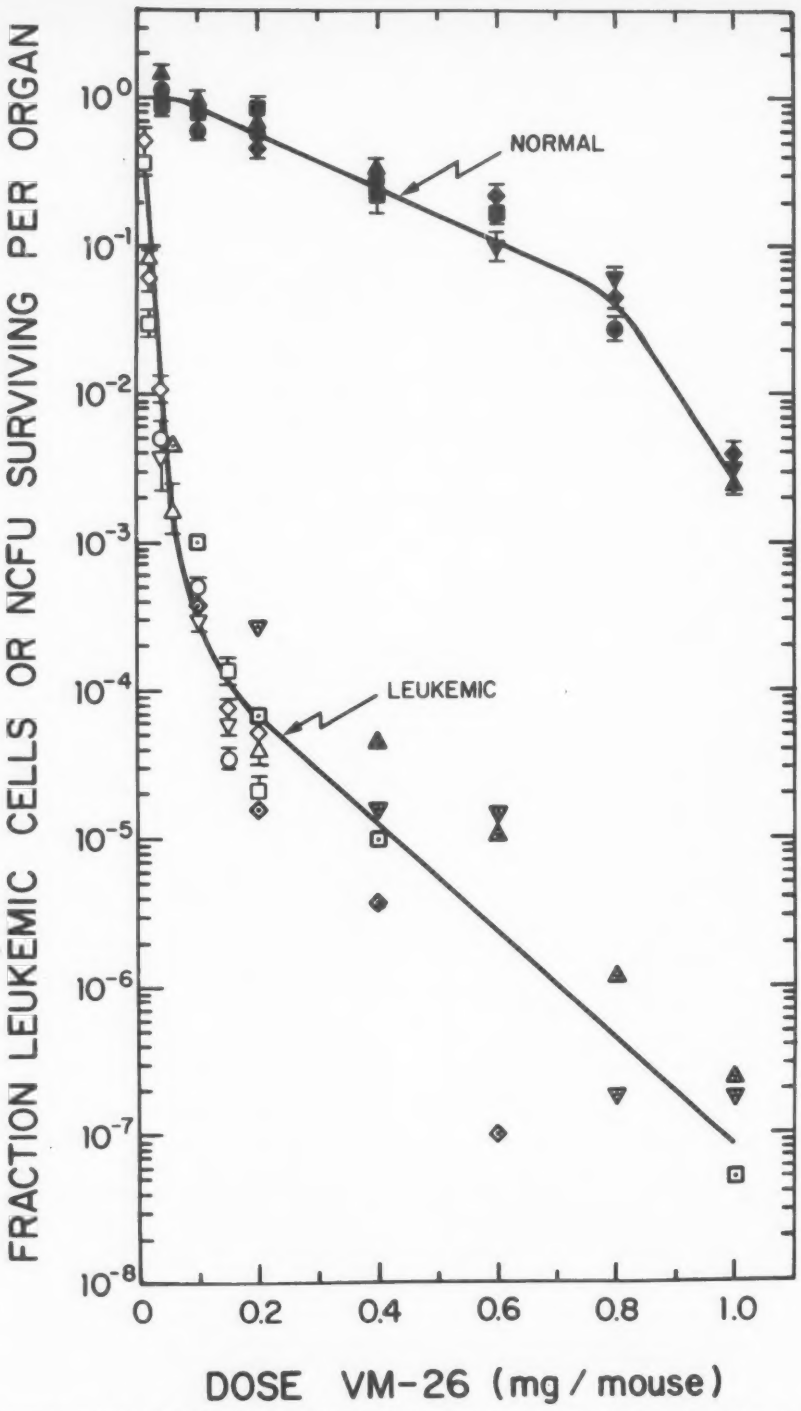

FIGURE 1.—Fractional survival of LCFU (open symbols) and NCFU (closed symbols) as a function of VM-26 dose. Symbols with dots = endpoint dilution assays. Different symbols = different experiments; errors are ± 1 SE.

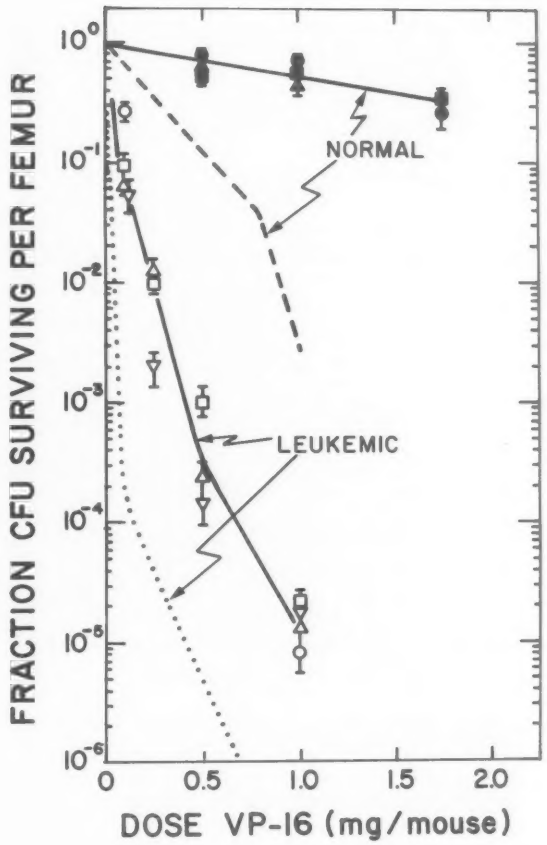

FIGURE 2.—Fractional survival of LCFU (open symbols) and NCFU (closed symbols) as a function of VP-16-213 dose. Results for VM-26 are shown for LCFU (dotted line) and NCFU (dashed line) taken from fig 1. Different symbols = different experiments; errors are ± SE.

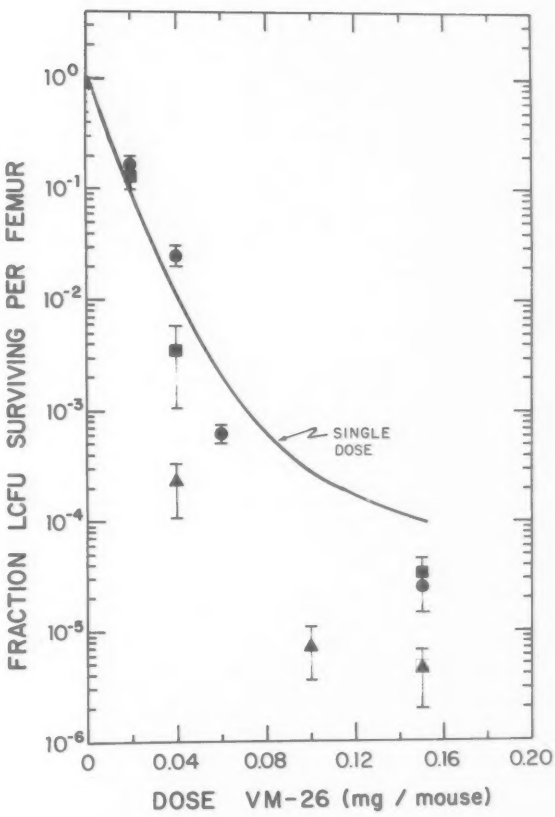

FIGURE 3.—Survival of LCFU as a function of dose of VM-26 administered as a 24-hour infusion. Solid line taken from fig 1 for rapid injection. Different symbols = different experiments; errors are ± 1 SE.

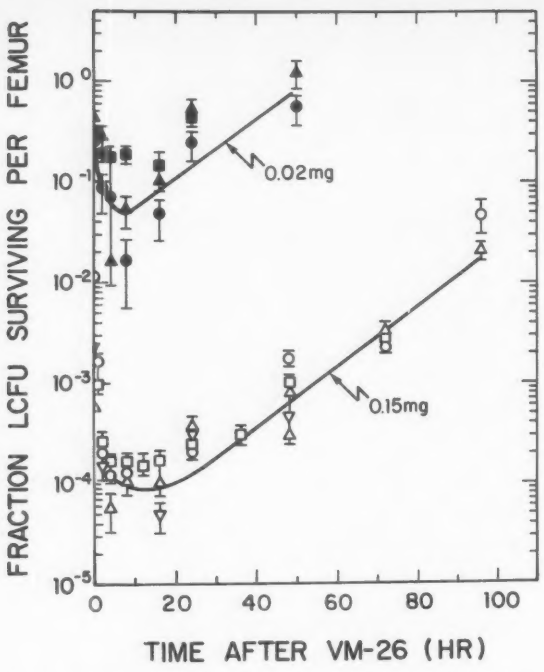

FIGURE 4.—Fractional survival of LCFU as a function of time after the administration of 0.05 mg/mouse (open symbols) and 0.15 mg/mouse (closed symbols) of VM-26. Different symbols = different experiments; errors are ± 1 SE.

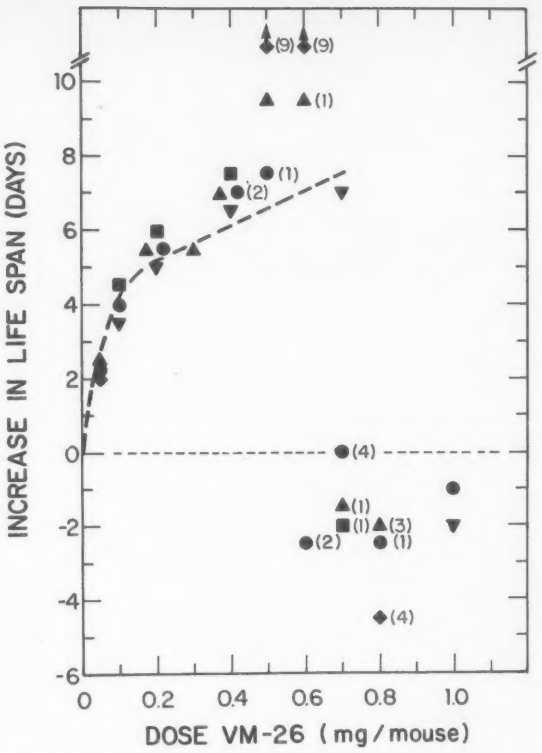

FIGURE 5.—ILS of leukemic mice as a function of VM-26 dose. Nos. in parentheses = 30-day survivors/10 treated animals. Different symbols = different experiments. Dashed line is expected from LCFU results as calculated in the text.

ogenic and the ILS assays. The assumption of no lag in LCFU proliferation following treatment is at variance with the data in figure 4, where it is noted that for 0.02 and 0.15 mg/mouse the delay in repopulation is 8 and 16 hours respectively. The expected discrepancy is estimated at 0.5 days for 0.15 mg and may explain the discrepancy between these two assays at doses > 0.3 mg/mouse.

Toxicity of VM-26

Seven separate experiments with ten mice per point and doses ranging from 0.1 to 1 mg/mouse were done for bolus iv injection. Dose increments of 0.1 mg/mouse were examined. The LD10, LD50 (with standard deviation), and LD90 values were calculated as 0.39, 0.51 (± 0.10), and 0.64 mg/mouse respectively. For the infusion studies, four separate experiments using five or ten mice per point were carried out at 0.1-mg dose increments over the same dose range as above. The LD10, LD50, and LD90 values were determined as 0.32, 0.47 (± 0.12), and 0.62 mg/mouse respectively.

DISCUSSION

One interesting result of these studies was the multiphasic form of the dose-survival curves for both VM-26 and VP-16-213; these curves indicate the existence of at least two populations of leukemia cells with different drug sensitivities. We have not examined the mechanism for the decreased sensitivity, but it may reflect either the appearance of drug-resistant mutants or the fraction of the cells not in the sensitive phase of the cell cycle. The initial slopes for both agents are quite steep and the values of 28 and 30 for differential cytotoxicity are the highest reported (26); a break occurs for both agents at a surviving fraction of 10^{-4}. The biphasic nature of these agents has been reported for NCFU (27). Except for high doses, cell-kill with infusions at a given dose yielded a similar response to bolus injection. The possibly enhanced cell-kill at higher doses agrees with schedule dependency studies which show that administration of the agent every

3 hours for 24 hours is the most effective (28). The toxicity in terms of animal survival was nearly identical for both schedules. The major toxicity in man is myelosuppression (29–31), but this seems inconsistent with our dose-survival curve for NCFU. For both VM-26 and VP-16-213, 0.6 mg/mouse results in no more than a 1-decade reduction in NCFU survival (figs 1 and 2); however, this is close to the LD50 dose (6,12). This may be due to a true interspecies difference in the toxicity of the agents or may point to a greater effect on the granulocytic progenitor cell compared to the hematopoietic stem cell with the former determining the toxicity (33).

The time-survival curves for low (0.02 mg/mouse) and high (0.15 mg/mouse) doses of VM-26 indicate that cell-killing occurs rapidly after drug administration and is of short duration. No further cell-killing was demonstrable 4 hours after drug administration. However, there did appear to be a dose-dependent lag in the repopulation lasting about 4 and 16 hours thereafter for the low and high dose respectively. This is presumably due to the fact that low doses of both VM-26 and VP-16-213 cause an inhibition of cell proliferation (2,6).

The ILS data were in excellent agreement with the results from the LCFU assay (fig 5) and agreed with those reported by Avery et al (14); both studies show a significant cure rate of mice in some experiments where mice received doses of 0.5–0.7 mg of VM-26 per mouse. This is expected since the mice harbor about 10^6 L1210 cells 2 days after the iv injection of 10^5 cells and a dose of 0.6 mg/mouse decreases survival of L1210 to about 10^{-6}; at greater doses, there is decreased lifespan due to host toxicity.

The therapeutic implications of this are: (*a*) repeated doses of drug equivalent to the level where the slope changes might prove optimal, and (*b*) combination therapy might be effective if the less sensitive population were still fully sensitive to a second agent. The former seems to have been demonstrated with a variety of fractionated schedules which appear effective; however, only a limited number of schedules have been examined (4,28,32). The latter possibility has also proven correct, especially in combination with cyclophosphamide or BCNU (20).

REFERENCES

1. KELLER-JUSLÉN C, KUHN M, STÄHELIN H, ET AL. Synthesis and antimitotic activity of glycosidic lignan derivatives related to podophyllotoxin. J Med Chem 14:936–940, 1971.
2. GRIEDER A, MAURER R, and STÄHELIN H. Effect of an epipodophyllotoxin derivative (VP-16-213) on macromolecular synthesis and mitosis in mastocytoma cells in vitro. Cancer Res 34:1788–1793, 1974.
3. MISRA NC, and ROBERTS D. Inhibition by 4′-demethyl-epipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) of human lymphoblast cultures in G_2 phase of the cell cycle. Cancer Res 35:99–105, 1975.
4. DREWINKO B, and BARLOGIE B. Survival and cycle-progression delay of human lymphoma cells in vitro exposed to VP-16-213. Cancer Treat Rep 60:1295–1306, 1976.
5. KRISHAN A, PAIKA K, and FREI E. Cytofluorometric studies on the action of podophyllotoxin and epipodophyllotoxins (VM-26, VP-16-213) on the cell cycle traverse of human lymphoblasts. J Cell Biol 66:521–530, 1975.
6. STÄHELIN H. 4′Demethyl-epipodophyllotoxin thenylidene glucoside (VM-26), a podophyllum compound with a new mechanism of action. Eur J Cancer 6:303–311, 1970.
7. LOIKE JD, and HOROWITZ SB. Effects of podophyllotoxin and VP-16-213 on microtubule assembly in vitro and nucleoside transport in HeLa cells. Biochemistry 15:5435–5443, 1976.
8. LOIKE JD, and HOROWITZ SB. Effect of VP-16-213 on the intracellular degradation of DNA in HeLa cells. Biochemistry 15:5443–5448, 1976.
9. HUANG CC, HOW Y, and WANG JJ. Effects of a new antitumor agent, epipodophyllotoxin, on growth and chromosomes in human hematopoietic cell lines. Cancer Res 33:3123–3129, 1973.
10. GOSALVEZ M, PEREZ-GARCIA J, and LOPEZ M. Letter to the Editor: Inhibition of NADH-linked respiration with the anticancer agent 4′-demethyl-epipodophyllotoxin thenylidene glucoside (VM-26). Eur J Cancer 8:471–473, 1972.
11. MUGGIA FM, SELAWRY OS, and HANSEN HH. Clinical studies with a new podophyllotoxin derivative, epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819). Cancer Chemother Rep 55:575–581, 1971.
12. STÄHELIN H. Activity of a new glycosidic lignan derivative (VP-16-213) related to podophyllotoxin in experimental tumors. Eur J Cancer 9:215–221, 1973.
13. NISSEN NI, LARSEN V, PEDERSEN H, ET AL. Phase I clinical trial of a new antitumor agent, 4′demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213). Cancer Chemother Rep 56:769–777, 1972.
14. AVERY TL, ROBERTS D, and PRICE RA. Delayed toxicity of 4′demethylepipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819; VM-26) in mice. Cancer Chemother Rep 57:165–173, 1973.
15. MATHÉ G, SCHWARZENBERG L, POUILLART P, ET AL. Two epipodophyllotoxin derivatives, VM 26 and VP 16213, in the treatment of leukemias, hematosarcomas, and lymphomas. Cancer 34:985–992, 1974.
16. EUROPEAN ORGANIZATION FOR RESEARCH ON THE TREATMENT OF CANCER, CLINICAL SCREENING GROUP. Epipodophyllotoxin VP-16-213 in treatment of acute leukaemias, haematosarcomas, and solid tumours. Br Med J 3:199–202, 1973.
17. EUROPEAN ORGANIZATION FOR RESEARCH ON THE TREATMENT OF CANCER, CO-OPERATIVE GROUP FOR LEUKAEMIAS AND HAEMATOSARCOMAS. Clinical screening of epipodophyllotoxin VM-26 in malignant lymphomas and solid tumours. Br Med J 2:744–748, 1972.
18. SKLANSKY BD, MANN-KAPLAN RS, REYNOLDS AF, JR, ET AL. 4′-Demethyl-epipodophyllotoxin-β-D-thenylidene-glucoside (PTG) in the treatment of malignant intracranial neoplasms. Cancer 33:460–467, 1974.
19. SONNTAG RW, SENN HJ, NAGEL G, ET AL. Experience with 4′-demethylepipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside); VM-26; NSC-122819 in the treatment of malignant lymphomas. Eur J Cancer 10:93–98, 1974.
20. DOMBERNOWSKY P, and NISSEN NI. Combination chemotherapy with 4′demethylepipodophyllotoxin 9-(4,6-*O*-ethyli-

dene-β-D-glucopyranoside), VP 16-213 (NSC 141540) in L1210 leukemia. Eur J Cancer 12:181–188, 1976.

21. VALERIOTE F, VIETTI T, and TOLEN S. Kinetics of the lethal effect of actinomycin D on normal and leukemic cells. Cancer Res 33:2658–2661, 1973.
22. WODINSKY I, SWINIARSKI J, and KENSLER CJ. Spleen colony studies of leukemia L1210. I. Growth kinetics of lymphocytic L1210 cells in vivo as determined by spleen colony assay. Cancer Chemother Rep 51:415–421, 1967.
23. BRUCE WR, and VAN DER GAAG H. A quantitative assay for the number of murine lymphoma cells capable of proliferation in vivo. Nature 199:79–80, 1963.
24. HEWITT HB. Studies of the dissemination and quantitative transplantation of a lymphocytic leukemia of CBA mice. Br J Cancer 12:378–401, 1958.
25. FISHER RA, and YATES F. Table VIII 2. *In* Statistical Tables for Biological, Agricultural and Medical Research, 6th ed. Darien, Conn, Hafner Publishing Co, 1963.
26. VALERIOTE FA, and TOLEN SJ. Survival of hematopoietic and lymphoma colony-forming cells in vivo following the administration of a variety of alkylating agents. Cancer Res 32:470–476, 1972.
27. VAN PUTTEN LM, SMINK T, and LELIEVELD P. Effects of spindle poisons on cell survival in the mouse. *In* Microtubules and Microtubule Inhibitors (Borgess M, and de Brabander M, eds). Amsterdam, North Holland Publishing Co, 1975, pp 523–532.
28. DOMBERNOWSKY P, and NISSEN NI. Schedule dependency of the antileukemic activity of the podophyllotoxin-derivative VP 16-213 (NSC-141540) in L1210 leukemia. Acta Pathol Microbiol Scand [A]81:715–724, 1973.
29. GOLDSMITH MA, and CARTER SK. 4'Demethyl-epipodophyllotoxin-β-D-thenylidene glucoside (VM-26)—a brief review. Eur J Cancer 9:477–482, 1973.
30. DOMBERNOWSKY P, NISSEN NI, and LARSEN V. Clinical investigation of a new podophyllum derivative, epipodophyllotoxin, 4'demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819), in patients with malignant lymphomas and solid tumors. Cancer Chemother Rep 56:71–82, 1972.
31. CREAVEN PJ, NEWMAN SJ, SELAWRY OS, ET AL. Phase I clinical trial of weekly administration of 4'demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213). Cancer Chemother Rep 58:901–907, 1974.
32. ROZENCWEIG M, VON HOFF DD, HENNEY JE, ET AL. VM 26 and VP 16-213: a comparative analysis. Cancer 40:334–342, 1977.
33. VALERIOTE F, and NAKEFF S. Effects of anticancer agents on hematopoietic progenitor cells. *In* The Year in Hematology. New York, Plenum Publishing Corp, 1978, vol 2. In press.

Comparative Disposition of Phosphoramide Mustard and Other Cyclophosphamide Metabolites in the Mouse Using the *Salmonella*/ Mutagenesis Assay [1,2]

William J. Suling,* Robert F. Struck, Carolyn W. Woolley, and William M. Shannon [3,4]

SUMMARY

The *Salmonella*/mutagenesis assay was used to compare the disposition of phosphoramide mustard (PM), an active metabolite of cyclophosphamide (CPA), and other mutagenic CPA metabolites in the mouse. A mutant strain of *Salmonella typhimurium*, TA-1535, which is reverted from a histidine requirement to prototrophy by mutagens causing DNA base-pair substitutions, was used to measure the mutagenic activity in tissues and urine of BDF_1 mice given single ip doses of PM (180 mg/kg) or CPA (342 mg/kg). Assuming that mutagenic activity in tissues and body fluids was due to unchanged drug and/or its metabolites, it was observed that PM was rapidly absorbed by the tissues and that drug levels were initially high in plasma, kidney, liver, and pancreas, but were not detectable in lung, spleen, and brain. These levels rapidly decreased with time. The apparent plasma half-life of PM was < 20 minutes. Mutagenic metabolites of CPA disappeared less rapidly from tissues than did PM. Peak levels of CPA metabolites in tissues were reached after 30 minutes and were high in plasma, liver, kidney, lung, spleen, and pancreas, but were not detectable in brain. The apparent plasma half-life of the CPA metabolites was 34 minutes. Approximately 34% of the dose of PM was recovered, as drug equivalents, in the urine within 6 hours after injection. Thin-layer chromatography of the urine of PM-treated mice indicated the presence of PM and *nor*-nitrogen mustard (*nor*-HN2). Urinary excretion of CPA and its metabolites was studied after injection of ^{14}C-CPA labeled in the sidechain. Metabolites in urine samples were differentially extracted with chloroform and methanol and were separated by thin-layer chromatography. Approximately 35% of the injected radioactivity was recovered in the urine within 6 hours. The major radiolabeled alkylating components in the urine samples were CPA, 4-keto-CPA, and *nor*-HN2. Minor components consisted of carboxyphosphamide and PM. The relative concentration of CPA excreted in the urine decreased with time; both 4-keto-CPA and *nor*-HN2 concomitantly increased with time. Mutagenic activity of the urine samples from CPA-injected mice correlated with the excretion of *nor*-HN2.

[Cancer Treat Rep 62:1321–1328, 1978]

The major pathway for the metabolism of cyclophosphamide (CPA) has recently been elucidated in an attempt to determine the biologically active metabolite responsible for its antitumor activity (1–4). Although 4-hydroxy-CPA and *nor*-nitrogen mustard (*nor*-HN2) are cytotoxic products of CPA metabolism, phosphoramide mustard (PM) is a prime candidate for the ultimate alkylating metabolite (5,6). Under physiologic conditions, this compound is an alkylating agent, acting via an aziridinium inter-

[1]Received Feb 22, 1978; revised June 19, 1978; accepted June 27, 1978.

[2]Supported by contract N01-CM-43780 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Ala.

[4]We wish to acknowledge the capable technical assistance of Diane Graveman and Louise Rice.

*Reprint requests to: Dr. William J. Suling, Kettering-Meyer Laboratory, Southern Research Institute, 2000 9th Ave S, Birmingham, Ala 35205.

mediate (6). It is directly toxic to cells (7), and measurable amounts are present in the plasma of patients given CPA (8). CPA and PM have similar activity in the chemotherapy of mice bearing L1210 leukemia (Schabel FM, Jr, unpublished results). The activity of PM against a variety of other animal tumor systems has also been demonstrated (9).

Assay methods for CPA and its metabolites have included radioisotopic, colorimetric (10), and mass-spectrometric techniques (11), as well as an in vivo bioassay with a transplantable mouse leukemia (12) and in vitro bioassays using cell cultures (13,14). We have been evaluating the utility of the *Salmonella*/mutagenicity test (15) for the assay of DNA-reactive antitumor agents, such as PM, in the urine and tissues of treated animals. The present study describes the use of this technique to compare the disposition of CPA with PM in BDF_1 mice.

MATERIALS AND METHODS

Chemicals

^{14}C-CPA (labeled in the sidechain) was obtained from Monsanto Research Corp, Dayton, Ohio, and was purified by thin-layer chromatography (TLC) on silica gel G plates with acetone:chloroform (3:1) solvent system. Specific activity of the purified material was 24 microcuries/mg. CPA and *nor*-HN2 were purchased from Mead Johnson, Evansville, Ind, and Aldrich Chemical Co, Miiwaukee, Wis, respectively. 4-Keto-CPA was synthesized as described elsewhere (16). PM (cyclohexylamine salt) was obtained from the National Cancer Institute, Bethesda, Md.

Mutagenicity Assay

Salmonella typhimurium LT2 strain TA-1535, which was kindly supplied by Dr. Bruce Ames, University of California, Berkeley, was used to assay for mutagenic activity. This strain is a histidine-requiring auxotroph which is reverted to prototrophy by mutagens which cause DNA base-pair substitutions (15). The assay was performed as recommended by Ames et al (15), except that the minimal medium contained 0.1% NH_4Cl, 0.73% K_2HPO_4, 0.3% KH_2PO_4, 0.012% $MgSO_4$, 0.4% glucose, and 1.5% agar; the pH was 7.0. Twenty ml of this medium was added to each assay plate (13 × 100-mm plastic Petri plate). Samples to be assayed (0.1 ml) were incorporated into the top agar (15) together with 0.1 ml of a 16-hour nutrient broth culture of TA-1535. All samples were assayed twice. The assay plates were incubated at 37°C for 48 hours, at which time the number of histidine-revertant colonies appearing on the plates was scored. Experimental controls included assay plates containing diluent, but no drug, as well as sterility controls for all of the assay components. The number of spontaneous revertants appearing on the diluent control plates was subtracted from the counts appearing on the test plates. Standard dose-response curves, obtained with serial twofold dilutions of PM (2.5–20 μg/plate) in 0.85% saline, plasma, mouse urine, and tissue extracts, were run for each experiment. These curves were used to convert the mutagenic activity (number of revertant colonies) to μg of PM equivalents.

Animal Studies

Male BDF_1 mice (20–22 g) were given single ip injections (0.5 ml) of CPA (342 mg/kg) or PM (180 mg/kg) dissolved in 0.85% saline. These were approximately LD10 doses. The historical LD10 doses of CPA and PM are 312 and 181 mg/kg respectively (Schabel FM, Jr, unpublished results). Control mice were injected with saline alone. At different times after injection, groups of five mice were killed by asphyxiation over dry ice, and blood was aseptically withdrawn (by cardiac puncture) into heparinized syringes, pooled in sterile centrifuge tubes, and held at 5°C. Blood cells were removed by centrifugation at 5°C and 1100 *g* for 10 minutes. The brain, liver, kidney, lung, spleen, and pancreas were also removed aseptically, rinsed with cold 0.85% saline, blotted on sterile filter paper, and weighed. The tissues were then homogenized in nine parts of cold saline in a Potter-Elvehjem apparatus with a Teflon pestle. Tissue homogenates were centrifuged at 5°C and 27,000 *g* for 45 minutes. The clear supernatants were pooled and stored frozen at –20°C until they were assayed for mutagenic activity. Urine was collected in tubes on ice via catheter from a group of ten to fifteen mice. The urine was frozen and stored at –20°C until it was assayed.

Urinary Metabolite Studies

Mice were injected ip with 342 mg/kg (0.05 microcurie/kg) of ^{14}C-CPA or with 180 mg/kg of PM. Urine samples were collected and handled as described above. Nonpolar and polar components in the urine from CPA-treated mice were differentially extracted with chloroform and methanol, separated by TLC, and measured as previously described (5). Urine samples from PM-treated mice were evaporated to dryness in vacuo, and the residues were suspended in methanol and then treated with excess ethereal diazomethane for 10 minutes with stirring. The resulting mixtures were filtered to remove solids

and the filtrates were evaporated to dryness over a stream of nitrogen at room temperature. The methylated urine extracts were finally dissolved in methanol to a volume equivalent to 10% of each initial urine sample. Separation of components of the mixtures was accomplished by TLC and alkylating components were detected with 4-(*p*-nitrobenzyl)pyridine and potassium hydroxide as previously described (5).

RESULTS

Mutagenicity Assay

The number of revertant colonies obtained with the urine, plasma, and tissue extracts of mice treated with CPA or PM was converted to PM equivalents using a standard dose-response curve for this compound. Standard curves were linear up to a PM concentration of 20 μg/assay plate. It was observed, however, that the mutagenic activity (revertant colonies/μg) of PM was affected by the diluent employed. Greater mutagenic activity was obtained when the drug was measured in extracts of kidney, liver, lung, spleen, and pancreas compared to extracts of brain, plasma, urine, or saline. It was necessary, therefore, to construct standard dose-response curves for PM diluted in each appropriate urine, plasma, and tissue extract. The number of spontaneous revertant colonies appearing on control plates varied from ten to 25. Control diluents had no apparent effect on the spontaneous revertant colony counts.

Animal Studies

The results of disposition studies of CPA and PM are presented in figures 1 and 2. PM and/or mutagenic products were rapidly absorbed by the tissues following ip injection of PM since peak levels were observed at 5 minutes, the earliest time of assay. Levels of drug equivalents were initially high in plasma, liver, kidney, and pancreas, but were below the limit of detection in the spleen, lung, and brain. No activity was detectable in the pancreas 15 minutes after injection. Mutagenic activity in the other tissues was below detectable levels within 60 minutes. The plasma half-life of PM equivalents

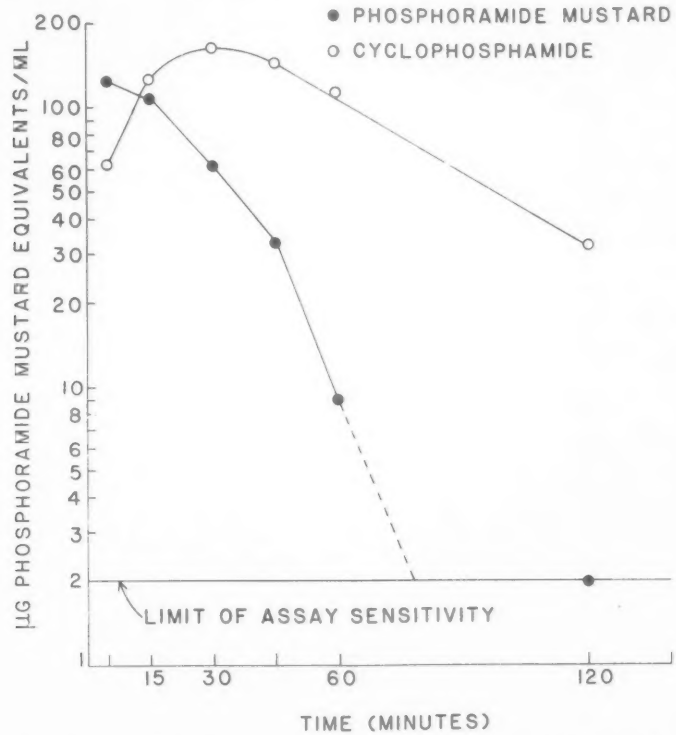

FIGURE 1.—Plasma levels of PM equivalents and mutagenic metabolites of CPA following a single ip injection of 180 and 342 mg/kg respectively. Each point on the curves was derived from the pooled plasma of 5 mice.

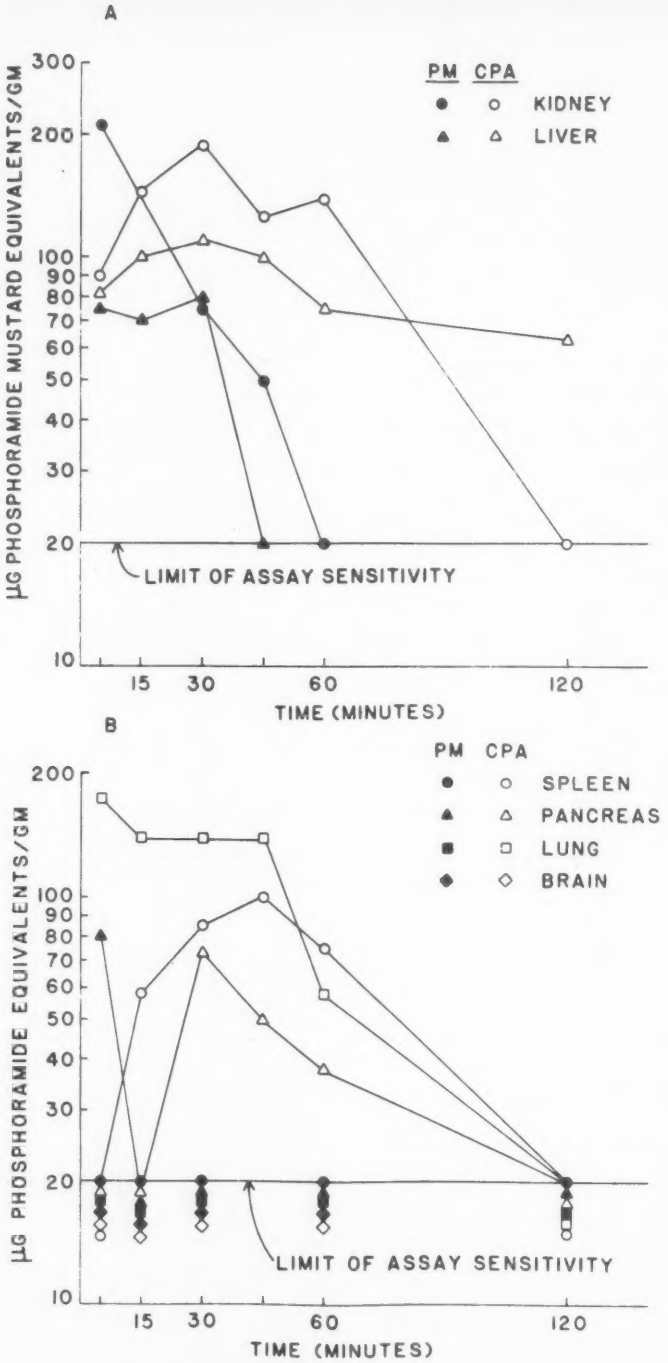

FIGURE 2.—Levels of PM equivalents (PM = 180 mg/kg) and mutagenic metabolites of CPA (CPA = 342 mg/kg) in kidney and liver (A) and spleen, pancreas, lung, and brain (B). Each point on the curves = the result of pooled tissue extracts from 5 mice. The limit of assay sensitivity line is the upper limit. All points falling on or below this line represent no detectable activity.

appeared to decrease with time but was < 20 minutes. Mutagenic metabolites of CPA disappeared less rapidly from the tissues than did PM. Peak levels of CPA metabolites in tissues were reached after 30 minutes and were high in plasma, liver, kidney, lung, spleen, and pancreas, but were not detectable in brain. The apparent plasma half-life of the CPA metabolites was 34 minutes.

Urinary excretion of PM equivalents of CPA and PM is shown in figure 3. The excretion of mutagens by mice treated with either drug increased continuously over the entire sampling period of 6 hours. Approximately 34% of the dose of PM, based upon PM equivalents, was recovered in the urine 6 hours after injection. PM and *nor*-HN2 were the only alkylating components detected in the urine after TLC. Based upon color intensity after 4-(*p*-nitrobenzyl)pyridine treatment of the chromatograms, it appeared that both PM and *nor*-HN2 were excreted in similar amounts for up to 3 hours, but that *nor*-HN2 was the predominant component excreted between 3 and 6 hours after injection.

Since it was likely that the mutagenic activity measured in the urine of mice treated with CPA was due to more than one metabolite, the excretion of ^{14}C-labeled CPA, as well as its major alkylating metabolites, was studied as described. Results based upon total counts per minute excreted are presented in figure 4. CPA, 4-keto-CPA, and *nor*-HN2 were major urinary components while carboxyphosphamide (CP) and/or PM represented minor components. Because of their low concentration, the latter two components were not separated. Approximately 35% of the injected radioactivity was recovered in the urine after 6 hours. The relative amounts of CPA and its major metabolites excreted with time are presented in figure 5. Relative levels of CPA decreased with time from 70% to 16%, while the levels of 4-keto-CPA and *nor*-HN2 concomitantly increased. The relative concentrations of CP + PM were never > 13% of the total. A comparison of the mutagenic activities of CPA and its metabolites, 4-keto-CPA and *nor*-HN2, with PM indicated that CPA was not mutagenic, that 4-keto-CPA was slightly mutagenic (0.2 revertant colonies/μg), and that *nor*-HN2 was more mutagenic (44 revertant colonies/μg) as compared to PM (13 revertant colonies/μg).

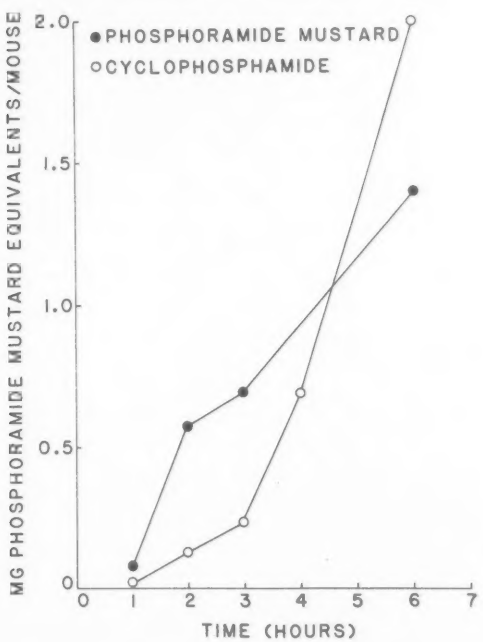

FIGURE 3.—Urinary excretion of PM equivalents and mutagenic metabolites of CPA. The mg PM equivalents excreted with time are cumulative values. Data were derived from the pooled urine of 10–15 mice.

DISCUSSION

The metabolism of CPA in the mouse was rapid since peak levels of mutagenic metabolites were observed within 30 minutes after treatment. The observation that high levels of CPA metabolites were present in all tissues examined, except the brain, is in agreement with studies performed by others using the mouse and other animal species (4,17). Following an injection of PM, the drug and/or its mutagenic products were rapidly absorbed and almost as rapidly eliminated from tissues. In contrast to the disposition of CPA metabolites, there was no detectable accumulation of mutagenic activity in the spleen or lung after PM treatment, and drug levels in the pancreas were below detectable levels within 15 minutes. The apparent low levels of activity in the spleen and lung could be due to poor permeability of these tissues to PM or to the rapid alkylation and binding of tissue components by the drug.

CP is the major urinary metabolite of CPA excreted by the dog (1), rat (18), sheep (19), and man (1), whereas 4-keto-CPA has been found to be a minor metabolite in these mammals when urine is collected for long periods after drug administration. The present study suggests that, for short collection periods (6 hours), the major urinary metabolites of CPA in the mouse are *nor*-HN2 and 4-keto-CPA. Relatively high levels of these two metabolites have also been observed in the blood of mice treated with CPA (5). More recent studies (20) suggest that significant levels of 4-hydroxy-CPA are present in

the blood of mice, rats, and man after CPA administration. Because of the instability of this metabolite, it was detectable in significant amounts only after derivatization with benzyl mercaptan to form the stable 4-(*S*-benzyl)mercapto-CPA. It is noteworthy to mention here that recent studies done by Jardine et al (21) suggest that some decomposition of CPA and its major metabolites to *nor*-HN2 can occur during the processing of plasma and urine prior to assay. Under the experimental conditions of the present study, it is possible that significant decomposition of CP and PM to *nor*-HN2 occurred after sampling and that the measured urine levels of *nor*-HN2 were artifactually high. Since 4-hydroxy-CPA would be unstable under our assay conditions, conversion of this metabolite to *nor*-HN2 is also possible. It should be kept in mind that the present study of urine levels of CPA and its major alkylating metabolites was performed in order to correlate metabolite levels with mutagenic activity. It appears that the mutagenic activity of urine and plasma, and presumably tissues, from mice treated with CPA was largely due to the presence of *nor*-HN2 since the other major metabolite, 4-keto-CPA, was shown to have only weak mutagenic activity in our assay system.

After an injection of PM, mice excreted both PM and *nor*-HN2 in the urine over the 6-hour collection period. These results correlate well with those of Struck et al (5) who analyzed the blood of mice treated with PM. These studies suggested the presence of PM, *nor*-HN2, and 3-(2-chloroethyl)-1,3-oxazolidin-2-one, a compound formed by the reaction of blood carbon dioxide with *nor*-HN2. Whether *nor*-

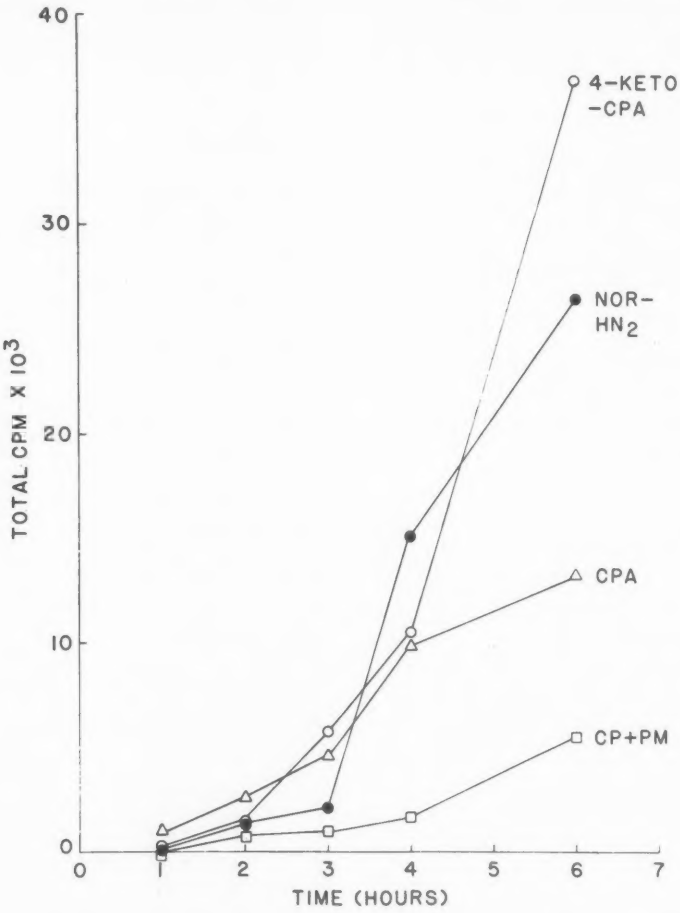

FIGURE 4.—Urinary excretion of ^{14}C-CPA and its major alkylating metabolites: 4-keto-CPA, *nor*-HN2, CP, and PM. The total counts per minute are cumulative values. Data were derived from the pooled urine of 15 mice.

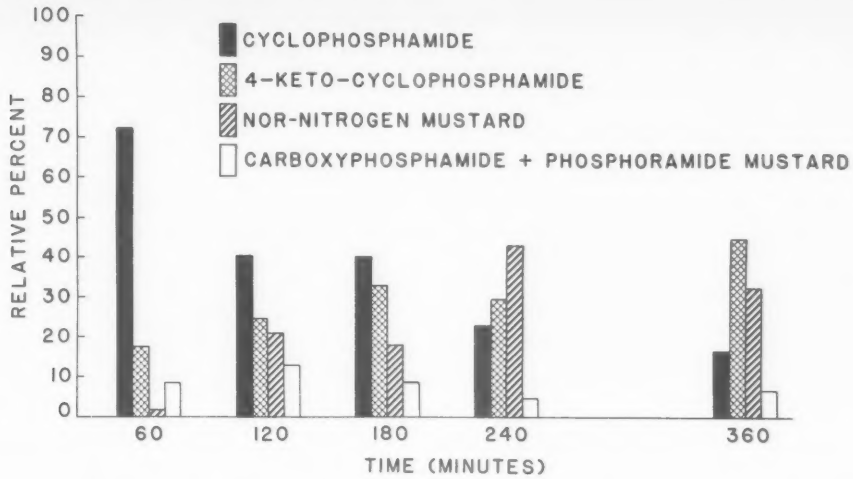

FIGURE 5.—Relative concentrations of CPA and alkylating metabolites excreted in the urine of mice injected with 342 mg/kg of ^{14}C-CPA. The values are cumulative. Data were derived from the pooled urine of 15 mice.

HN2 is formed spontaneously or enzymatically in the mouse is not known. According to these results, one can conclude that the mutagenic activity of the urine and plasma, and presumably other tissues, from PM-treated mice was due primarily to both PM and *nor*-HN2, although the present studies do not exclude the formation of additional mutagenic products.

The weak mutagenic activity of 4-keto-CPA is in agreement with its reported weak alkylating and cytotoxic activity (22). The greater mutagenic activity of *nor*-HN2 over that of PM is interesting since, at physiologic pH, PM has been reported to be the better alkylating agent (5,6). Both compounds were reported to have similar activity against H.Ep-2 cells, but PM was more active against KB cells (5).

The microbial mutagenicity assay used in this study may prove useful for the assay of other anticancer agents which are also mutagenic. It offers an advantage over colorimetric assays which are based upon alkylating activity because it is a direct determination of biologic activity, whereas the alkylating test, as normally applied (10), does not differentiate adequately between chemical and biologic activity. Coupled with TLC or paper chromatography, the mutagenicity assay could provide a convenient technique for quantitative determination of relative amounts of parent drug and metabolites in various tissues and therefore circumvent the preparation of a radioactive sample. This concept has recently been applied to the detection of mutagenic nitrofuran compounds and their metabolites (23).

REFERENCES

1. STRUCK RF, KIRK MC, MELLETT LB, ET AL. Urinary metabolites of the antitumor agent cyclophosphamide. Mol Pharmacol 7:519–529, 1971.
2. HILL DL, LASTER WR, and STRUCK RF. Enzymatic metabolism of cyclophosphamide and nicotine and production of a toxic cyclophosphamide metabolite. Cancer Res 32:658–665, 1972.
3. COLVIN M, PADGETT CA, and FENSELAU C. A biologically active metabolite of cyclophosphamide. Cancer Res 33:915–918, 1973.
4. HILL DL. A Review of Cyclophosphamide. Springfield, Charles C. Thomas, 1975.
5. STRUCK RF, KIRK MC, WITT MH, ET AL. Isolation and mass spectral identification of blood metabolites of cyclophosphamide: evidence for phosphoramide mustard as the biologically active metabolite. Biomed Mass Spectrom 2:46–52, 1975.
6. COLVIN M, BRUNDRETT RB, KAN M-NN, ET AL. Alkylating properties of phosphoramide mustard. Cancer Res 36:1121–1126, 1976.
7. MADDOCK CL, HANDLER AH, FRIEDMAN OM, ET AL. Primary evaluation of alkylating agent cyclohexylamine salt of *N,N*-bis(2-chloroethyl)phosphorodiamidic acid (NSC-69945; OMF-59) in experimental antitumor assay systems. Cancer Chemother Rep 50:629–639, 1966.
8. FENSELAU C, KAN M-NN, BILLETS S, ET AL. Identification of phosphorodiamidic acid mustard as a human metabolite of cyclophosphamide. Cancer Res 35:1453–1457, 1975.
9. FRIEDMAN OM, WODINSKY I, and MYLES A. Cyclophosphamide (NSC-26271)-related phosphoramide mustards—recent advances and historical perspective. Cancer Treat Rep 60:337–346, 1976.
10. FRIEDMAN OM, and BOGER E. Colorimetric estimation of nitrogen mustards in aqueous media. Hydrolytic behavior of bis(2-chloroethyl)amine, *nor*-HN2. Anal Chem 33:906–910, 1961.
11. SCHULTEN H-R. Qualitative and quantitative investigations of cyclophosphamide (NSC-26271), cyclophosphamide metabolites, and related compounds by field-desorption mass spectrometry. Cancer Treat Rep 60:501–507, 1976.

12. VENDITTI JM, HUMPHREYS SR, and GOLDIN A. The effectiveness of Cytoxan against mouse leukemia L1210 and resistant sublines. Cancer Chemother Rep 3:6–8, 1959.
13. WHEELER GP, BOWDON BJ, ADAMSON DJ, ET AL. Effects of certain nitrogen mustards upon the progression of cultured H.Ep. No. 2 cells through the cell cycle. Cancer Res 30:100–111, 1970.
14. LAUX D, and LAUSCH RN. In vitro microassay for biological activity of cyclophosphamide metabolites. Antimicrob Agents Chemother 3:407–411, 1973.
15. AMES BN, MCCANN J, and YAMASAKI E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat Res 31:347–364, 1975.
16. HOHORST H-J, PETER G, and STRUCK RF. Synthesis of 4-hydroperoxy derivatives of ifosfamide and trofosfamide by direct ozonation and preliminary antitumor evaluation in vivo. Cancer Res 36:2278–2281, 1976.
17. TORKELSON AR, LABUDDE JA, and WEIKEL JH, JR. The metabolic fate of cyclophosphamide. Drug Metab Rev 3:131–165, 1974.
18. VOELCKER G, WAGNER T, and HOHORST H-J. Identification and pharmacokinetics of cyclophosphamide (NSC-26271) metabolites in vivo. Cancer Treat Rep 60:415–422, 1976.
19. BAKKE JE, FEIL VJ, FJELSTUL CE, ET AL. Metabolism of cyclophosphamide by sheep. J Agric Food Chem 20:384–388, 1972.
20. WAGNER T, PETER G, VOELCKER G, ET AL. Characterization and quantitative estimation of activated cyclophosphamide in blood and urine. Cancer Res 37:2592–2596, 1977.
21. JARDINE I, FENSELAU C, APPLER M, ET AL. Quantitation by gas chromatography-chemical ionization mass spectrometry of cyclophosphamide, phosphoramide mustard, and nornitrogen mustard in the plasma and urine of patients receiving cyclophosphamide therapy. Cancer Res 38:408–415, 1978.
22. BROCK N. Comparative pharmacologic study in vitro and in vivo with cyclophosphamide (NSC-26271), cyclophosphamide metabolites, and plain nitrogen mustard compounds. Cancer Treat Rep 60:301–308, 1976.
23. HAYASHIDA S, WANG CY, and BRYAN GT. A simple method for detection of carcinogenic nitrofuran compounds and their metabolites by combining chromatography and spot mutation tests. Gann 67:617–619, 1976.

Estramustine Phosphate in the Treatment of Advanced Malignant Melanoma [1]

Reynold Lopez,[2,*] Constantine P. Karakousis,[2] Mukund S. Didolkar,[3] and E. Douglas Holyoke [2]

SUMMARY

Twenty-six patients (14 men and 12 women) with histologically proven advanced malignant melanoma, who previously had not responded to DTIC, methyl-CCNU, and procarbazine, received estramustine phosphate (Estracyt) (15 mg/m²) in daily divided doses. All patients had measurable disease. One patient developed a complete remission and one patient had improvement in liver function without measurable regression in the tumor. In three other patients (11%), the disease remained static for a period of 3–5 months. The mean survival time from the beginning of therapy was 16.8 months for the patients with a response or static disease and 2.18 months for those who had no response. Gastrointestinal toxicity was minimal; no hematologic toxicity was observed. It appears that estramustine phosphate used as a single agent for treating advanced malignant melanoma after patients failed to respond to DTIC and to the combination of methyl-CCNU and procarbazine has a poor response rate.

[Cancer Treat Rep 62:1329–1332, 1978]

Malignant melanoma is a rapidly growing, highly malignant tumor; < 5% of patients with advanced disease have a 5-year survival (1). Chemotherapy which gives only transient palliation is the main treatment for advanced metastatic disease. DTIC is the most commonly used chemotherapeutic agent, as a single agent or in combination with other agents; the response rate is in the range of 20%–30% (2). Other chemotherapeutic agents have not significantly altered the survival rate. Because of multiple organ involvement, ablative surgery or radiation therapy is of little help to these patients. There is a great need for more effective chemotherapeutic agents.

The purpose of this study was to evaluate the use of estramustine phosphate (Estracyt) in patients with advanced malignant melanoma who had previously failed to respond to DTIC therapy and to combination therapy with methyl-CCNU and procarbazine.

Estramustine phosphate is a hormone (estrogen) bonded to a nitrogen mustard (mechlorethamine), a known chemotherapeutic agent. Chemically, it might be considered an orethane-type nitrogen mustard. Its activation depends upon enzymes which activate the alkylating moiety. Ideally, the steroid moiety is the vehicle to direct the molecule into hormonally influenced tumors where the alkyl group would be activated (3). It is relatively nontoxic and has shown some beneficial effects in patients with advanced prostatic carcinoma (4).

MATERIALS AND METHODS

Twenty-six patients (14 men and 12 women) with histologically proven advanced malignant melanoma were studied. The age range was from 24 to 74 years, with an average of 55 years. All patients had failed to respond to treatment with DTIC, methyl-CCNU, and procarbazine. A 6-week interval without chemotherapy prior to the start of estramustine phosphate therapy and no previous therapy with estrogen-binding compounds were required. The patient's minimum life expectancy had

[1]Received Apr 10, 1978; revised June 21, 1978; accepted July 10, 1978.

[2]Department of Surgical Oncology, New York State Department of Health, Roswell Park Memorial Institute, Buffalo.

[3]Department of Surgery, Veterans' Administration Hospital, Sioux Falls, SD.

**Reprint requests to:* Reynold Lopez, MD, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

TABLE 1.—Site of metastases*

Site	No. of patients	%
Skin	19†	63
Subcutaneous	20†	76
Liver	9‡	35
Lung	12	46
Bone	2	8
Brain	4	15
Gastrointestinal	1	3
Nodes	12	46

*Frequently multiple metastases were present.
†Patient with CR.
‡PR (decreased liver enzymes).

to be > 4 weeks. Measurable disease was present, and hepatomegaly was considered a measurable disease when the liver was palpated at least 5 cm below the right costal margin. All patients had multiple metastases as shown in table 1.

The standard Eastern Cooperative Oncology Group performance status was grade 1 in one patient, grade 2 in three, grade 3 in 17, and grade 4 in five.

The prior chemotherapy which had been given to these patients can be summarized as follows. Most patients who received DTIC received *Corynebacterium parvum* along with DTIC. Methyl-CCNU and procarbazine were given as a second line of treatment. No patients had received radiation therapy. After treatment failure, 15 mg/m²/day of estramustine phosphate was administered orally in a divided dose. Prochlorperazine (Compazine) was given for nausea and vomiting. Blood cell counts were done before the treatment was started and were repeated once every week for the first 3 weeks of each treatment course. All patients had roentgenologic examination of the chest; in addition, radiologic examination of the stomach and small bowel and radioisotopic examination of the liver and brain were done in order to evaluate the extent of the disease. Blood chemistries, including alkaline phosphatase, LDH, SGOT, bilirubin, and BUN, were evaluated at the beginning of the treatment and monthly thereafter.

Patients were evaluated at 4-week intervals, at which time toxic effects were also recorded.

A complete response (CR) was defined as the complete disappearance of disease for at least 6 weeks. A partial response (PR) was shown by a decrease of at least 50% in the product of two perpendicular diameters of the measurable lesions lasting for at least 6 weeks and no new lesions. Static disease was indicated by stabilization in the growth of measurable lesions for at least 6 weeks and no new lesions. Progression was evidenced by an increase of ≥ 25% in the product of two perpendicular diameters of the measurable lesions and/or the development of new lesions.

A PR of liver metastases was defined as a reduction by at least 50% of the product of the liver measurements below the xiphoid and costal margins at the midclavicular line, while the liver function tests remained stable or improved.

RESULTS

One of the 26 patients treated had a CR and has remained free of disease for 3 years. Her lesions were in the subcutaneous tissues and skin of the left thigh, anterior chest, and upper abdomen. There was one patient with massive liver metastasis who showed a significant decrease in the elevated liver enzymes. Although neither the physical examination nor the liver scan showed any evidence of improvement, the disease remained under control for 4 months. Both of these patients were women.

Three other patients (11.5%), two men and one woman, had visceral and subcutaneous lesions that remained static for 6 months. In the remainder of the patients (80.8%), the tumor progressed in spite of the estramustine phosphate therapy. No patient who had previously responded to DTIC or to methyl-CCNU and procarbazine showed any response to estramustine phosphate.

The mean survival time of patients with CR, PR, and static disease was 16.8 months. The mean survival time of those patients who did not show any response was only 2.18 months.

Toxicity

There was no evidence of myelosuppression and no patient died due to the treatment. Gastrointestinal toxic effects in the form of nausea and vomiting were seen in only eight patients; in another two patients the nausea and vomiting was severe enough to discontinue treatment (table 2).

There were four instances of weight gain and only one instance of anemia. There was no evidence of hepatic toxicity and no evidence of gynecomastia in the treated male patients.

DISCUSSION

Although DTIC is the most widely used agent in the treatment of advanced malignant melanoma, a large group of patients with tumors resistant to

TABLE 2.—Summary of oral estramustine phosphate toxicity in 26 patients

Toxic effect	No. of patients
Transient nausea	8
Nausea and vomiting requiring discontinuation of therapy	2
Fluid retention	4
Hematologic	1
Hepatic	0
Gynecomastia in men	0

DTIC therapy need other more effective chemotherapeutic regimens (2,5).

In our previously reported experience, the combination of methyl-CCNU and procarbazine showed a very low response rate (6).

In this study, an attempt was made to improve the survival of advanced melanoma patients by using a drug which consists of a combination of an estrogen bonded to a nitrogen mustard. Preliminary results of this trial have been published (7).

The rationale for testing the effect of estramustine phosphate in malignant melanoma is based on the superior response rate and survival of women with melanoma when compared to men as discussed by Nathanson et al (8) and Luce (1). Recent evidence has shown cytoplasmic estrogen-receptor activity in biopsy specimens of 40% of patients with malignant melanoma (9). In 1973, it was postulated that human malignant melanoma is an estrogen-dependent tumor, similar in its behavior to carcinoma of the breast, kidney, and endometrium (10). The evidence presented consisted of case reports showing activation of melanoma after estrogen administration. As supporting evidence, Sadoff et al (10) found a higher incidence of melanoma in women in their 20s and 40s; the former represents the peak age of childbearing activity and use of oral contraceptives, and the latter represents menopause. Animal work by Snyder and Das Gupta has shown that hamster melanoma has some estrogen-receptor activity and that its growth is enhanced by exogenous estrogen (11).

All of our patients had previously failed twice to respond to combination chemotherapy. It is well known that the response rate of a malignant tumor to a certain chemotherapeutic regimen decreases considerably when the latter is used as secondary rather than primary treatment. Similar results using DTIC and vincristine followed by methyl-CCNU have been published by other investigators (12). The poor response of estramustine phosphate described herein may thus be partly due to the advanced stage of disease in most patients and failure to respond to two previous regimens of chemotherapy. The patients in this series who originally responded to DTIC, later failed to respond to the combination of methyl-CCNU and procarbazine and also to estramustine phosphate alone.

The patient with the CR had metastatic disease to the subcutaneous tissue of the chest, thigh, and abdomen which is indicative of hematogenous spread. Only one of our patients with visceral disease showed any sign of improvement when treated with estramustine phosphate and this was a questionable PR of short duration. Other investigators have already shown that when metastases are visceral, the response rates to chemotherapy for malignant melanoma are poor (13).

The toxicity seen with estramustine phosphate administration was minimal and different from other chemotherapeutic agents. We did not see evidence of myelosuppression, making it an ideal candidate for combination chemotherapy. At present, we believe that estramustine phosphate has only a limited place in the treatment of advanced malignant melanoma when used after failure to respond to other chemotherapeutic agents. However, it should be given a trial as primary single-drug therapy, or as one drug in a combination, especially in those patients with estrogen-receptor positive melanomas. At present, we are evaluating our patients with regard to estrogen-receptor protein.

REFERENCES

1. LUCE JK. Chemotherapy of malignant melanoma. Cancer 30:1604–1615, 1972.
2. COMIS RL, and CARTER SK. Integration of chemotherapy into combined modality therapy of solid tumors. IV. Malignant melanoma. Cancer Treat Rev 1:285–304, 1974.
3. HOGBERG B. Chemistry and Pharmacology of Estracyt. Helsingborg, Sweden, U.P.J. Estracyt Peo, May 1973.
4. MUNTZING J, SHUKLA SK, CHU TM, ET AL. Pharmacological study of oral estramustine phosphate (Estracyt) in advanced carcinoma of the prostate. Invest Oncol 2:65–68, 1974.
5. GERNER RE, MOORE GE, and DIDOLKAR MS. Chemotherapy of disseminated malignant melanoma with dimethyl triazeno imidazole carboxamide (DTIC) and dactinomycin. Cancer 32:756–760, 1973.
6. DIDOLKAR MS, BAFFI RR, CATANE R, ET AL. Letter: Use of methyl-CCNU and procarbazine in advanced malignant melanoma resistant to DTIC therapy. Cancer Treat Rep 61:1738–1739, 1977.
7. DIDOLKAR MS, CATANE R, LOPEZ R, ET AL. Estramustine phosphate (Estracyt) in advanced malignant melanoma resistant to DTIC treatment. Proc Am Assoc Cancer Res and ASCO 19:381, 1978.
8. NATHANSON L, HALL TC, and FARBER S. Biological aspects of human malignant melanoma. Cancer 20:650–655, 1967.

9. FISHER RI, NEIFELD JP, and LIPPMAN ME. Oestrogen receptors in human malignant melanoma. Lancet 2(7981):337–339, 1976.
10. SADOFF L, WINKLEY J, and TYSON S. Is malignant melanoma an endocrine-dependent tumor? Oncology 27:244–257, 1973.
11. SNYDER J, and DAS GUPTA TK. Estrogen receptors in hamster melanoma, HM MEL1. (Abstr) Fed Proc 36:349, 1977.
12. AHMANN DL, HAHN RG, and BISEL HF. Evaluation of 1-(2-chloroethyl-3-4-methylcyclohexyl)-1-nitrosourea (methyl-CCNU, NSC 95441) versus combined imidazole carboxamide (NSC 45388) and vincristine (NSC 67574) in palliation of disseminated malignant melanoma. Cancer 33:615–618, 1974.
13. EINHORN LH, BURGESS A, VALLEJOS C, ET AL. Prognostic correlations and response to treatment in advanced metastatic malignant melanoma. Cancer Res 34:1995–2004, 1974.

Phase I Trial of Vindesine in Patients With Advanced Cancer [1,2,3]

Violante E. Currie,* Peter P. Wong, Irwin H. Krakoff, and Charles W. Young [4]

SUMMARY

A phase I evaluation of vindesine was carried out in 69 adult patients with advanced malignancies. Two escalating dose schedules were explored: (*a*) a single dose every 7–14 days, and (*b*) daily injections × 5–10 days as tolerated. The main toxic effects were myelosuppression, alopecia, paresthesia, asthenia, myalgia, and hyporeflexia. Antitumor activity was seen during this phase I study in patients with leukemia, lymphoma, and testicular neoplasms. Disease oriented phase II trials of 3–4 mg/m² every 7–14 days or 1.3–2.0 mg/m²/day × 5–7 days every 3 weeks would be appropriate.

[Cancer Treat Rep 62:1333–1336, 1978]

Vindesine (desacetyl vinblastine amide sulfate) is a chemically derived structural analog of the vinca alkaloid, vinblastine sulfate (fig 1). Its antineoplastic activity against transplanted tumors in rodents more closely resembles that of another vinca alkaloid, vincristine sulfate, than it does that of vinblastine (1,2). Vindesine entered clinical trial because of the potential merit present in analogs of clinically useful compounds. In particular, it was hoped that the enlarged antitumor spectrum of this vinblastine analog in animals might presage a similarly increased therapeutic range in man. Preliminary abstracts on the phase I clinical trials have been published (3,4); this paper describes our observations in detail.

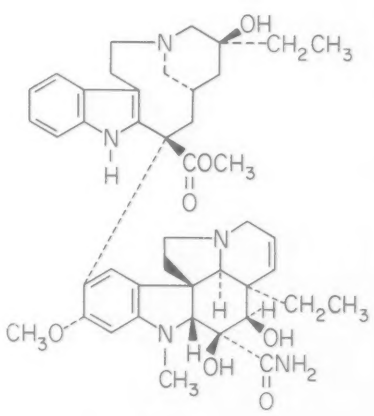

FIGURE 1.—Structure of vindesine.

PATIENTS AND METHODS

Vindesine was supplied by Eli Lilly and Co, Indianapolis, Ind. Sterile ampules containing 10 mg of vindesine were dissolved in a diluent containing sodium chloride, benzyl alcohol, and water for injection. The medication was mixed just prior to use at a concentration of 1 mg/ml and was injected iv. All patients selected for this trial had a firmly established diagnosis of advanced cancer for which no effective conventional therapy was available. Most of the patients had received prior therapy with vinca alkaloids. Informed consent for the drug trial was obtained from all patients.

This report will relate results with two dosage schedules, (*a*) a single dose every 7–14 days, and (*b*) daily injections × 5–10 days as tolerated. The weekly dose was developed first because of prior

[1]Received June 27, 1977; revised July 5, 1978; accepted July 12, 1978.

[2]Presented in part at the 7th Annual Meeting of The American Association for Cancer Research, Toronto, Canada, May 4–8, 1976.

[3]Supported in part by Public Health Service grants CA-08747 and CA-05826 and contract N01-CM-43804 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[4]Clinical Chemotherapy Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

**Reprint requests to:* Violante E. Currie, MD, Clinical Chemotherapy Service, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

TABLE 1.—Clinical side effects and toxicity of single-dose and daily dose vindesine therapy

Side effect	No. of patients— At risk: Single-dose	At risk: Daily dose	With toxic effects (%): Single-dose	With toxic effects (%): Daily dose	Total % with toxic effects
Alopecia	45	17	14 (31)	8 (47)	35
Paresthesia	45	23	15 (33)	11 (48)	38
Asthenia	45	24	14 (31)	7 (29)	30
Myalgia	45	24	0	6 (25)	8
Hyporeflexia	45	0	14 (26)	—	26
Muscle spasm	45	24	6 (13)	0	8
Pyrexia	45	18	4 (8)	8 (44)	19
Constipation	45	24	2 (4)	5 (21)	10
Paralytic ileus	45	24	0	5 (21)	7
Diarrhea	45	24	5 (11)	1 (4)	8
Anorexia	45	24	5 (11)	1 (4)	8
Nausea/vomiting	45	24	3 (6)	2 (8)	7
Stomatitis	45	24	2 (4)	7 (29)	13
Jaw pain	45	24	4 (8)	1 (4)	7
Photophobia	45	24	0	2 (4)	3

experience that both vinblastine and vincristine can be administered with therapeutic benefit by a weekly schedule. A daily iv schedule was developed at this Center by Dr. Charlotte Tan and colleagues of the Department of Pediatrics. The single-dose regimen was extended to adults with rapidly proliferating neoplastic disease because it had produced remissions in children with acute leukemia (Tan C, unpublished data).

Dosage

The phase I evaluation was initiated at a single-dose level of 0.5 mg/m^2 and was gradually escalated both in a given patient and within the patient group until a biologically active level was reached. Because vindesine had induced remission in children at a dose of 2 mg/m^2/day × 5 days, the adult schedules examined daily doses of 1.0, 1.3, 1.6, and 2.0 mg/m^2/day. Two to three weeks elapsed between courses of therapy.

Evaluation Criteria

A patient was considered evaluable at a given dose level if he manifested toxic effects, received two weekly injections, or completed a course of therapy (daily doses × 5 days). Complete remission requires complete disappearance of all evidence of disease with an improved (or normal) performance status for > 1 month. A partial remission is defined as a > 50% reduction of the product to two diameters of all measurable disease accompanied by significant clinical improvement for ≥ 1 month. A minor response was defined as unequivocal evidence of anticancer effect which was not of clinical benefit either by reason of its lower magnitude or short duration, or by the concomitant presence of severe drug toxicity which prevented improvement in performance status.

RESULTS AND DISCUSSION

Phase I evaluation of vindesine has been carried out in 45 adults by single-dose administration and in 24 adults by daily administration. The subjective and objective physical manifestations of drug toxicity are summarized in table 1. Although many of the patients treated with a single dose of 4.0 mg/m^2 or with a daily dose of ≥ 1.6 mg/m^2 had a profound sense of asthenia and malaise, these symptoms were dose-limiting in only two patients treated with the single dose of 4.0 mg/m^2. Significant pain, similar to myalgia rather than the neuralgic dysesthesia commonly observed with iv colchicine or vincristine, was only seen in patients treated with the daily schedule at a dose of ≥ 1.6 mg/m^2/day × 5–7 days. Constipation rarely occurred during weekly therapy (4% of the patients), but was more common with daily therapy (21% of the patients). No patient receiving single-dose therapy developed paralytic ileus but five patients receiving daily therapy developed this complication. Pyrexia and stomatitis were more common with the daily schedule. Drug tolerance was better in patients < 40 years of age and was significantly worse in patients > 60 years of age. With one exception, patients with severe liver dysfunction were not entered in this trial because of previous observations that they had not tolerated other vinca alkaloids satisfactorily.

TABLE 2.—Hematopoietic toxicity of single-dose vindesine therapy: dose-effect relationships

Dose (mg/m²)	No. of patients at risk	No. of evaluable courses	No. of courses with wbc nadir (× 10³/mm³) of—			No. of patients at risk	No. of evaluable courses	No. of courses with platelet nadir (× 10³/mm³) of—		
			> 4.0	2.0–3.9	< 2.0			> 150	80–150	< 80
0.5	2	4	3	1	0	2	4	3	0	1
1.0	3	4	3	1	0	3	4	3	1	0
2.0	10	11	5	5	1	10	11	6	3	2
3.0	12	19	6	8	5	12	17	13	2	2
4.0	33	54	26	14	14	33	40	29	7	4

TABLE 3.—Hematopoietic toxicity of daily dose vindesine therapy: dose-effect relationships

Dose (mg/m²)	No. of patients at risk	No. of evaluable courses	No. of courses with wbc nadir (× 10³/mm³) of—			No. of patients at risk	No. of evaluable courses	No. of courses with platelet nadir (× 10³/mm³) of—		
			> 4.0	2.0–3.9	< 2.0			> 150	80–150	< 80
1.0 × 5	12	15	4	4	7	12	9	8	1	0
1.3 × 5	11	15	4	2	9	11	12	5	6	1
1.6 × 5	5	4	0	0	4	5	2	0	2	0
2.0 × 5	7	7	1	0	6	7	4	1	1	2

TABLE 4.—Vindesine myelosuppression

	Single-dose regimen			Daily dose regimen		
	No. of courses	Median treatment day		No. of courses	Median treatment day	
		Nadir	Recovery		Nadir	Recovery
Leukopenia						
Mild*	29	7	11	6	11	18
Severe†	20	8	13	26	13	16
Thrombocytopenia						
Mild*	13	6	10	10	9	13
Severe†	9	7	12	3	8	13

*Wbc nadir ≥ 2.0 × 10³/mm³, platelet nadir ≥ 80 × 10³/mm³.
†Wbc nadir < 2.0 × 10³/mm³, platelet nadir < 80 × 10³/mm³.

The dose-effect relationships between the quantity of drug administered and hematopoietic toxicity are summarized in tables 2 and 3. Table 4 illustrates the time of nadir and recovery from these side effects. With rare exceptions, the wbc depression was more common and more severe than the platelet depression. The nadirs of leukopenia and thrombocytopenia on the single-dose schedule were essentially the same (7–8 and 6–7 days respectively), with recovery also being equal (11–13 and 10–12 days respectively).

On the daily schedule, the nadir and recovery of the platelet count occurred earlier than the nadir and recovery of the wbc count (platelet nadir, 7.5–9 days with recovery at 12.5 days; wbc nadir, 11–12.5 days with recovery at 16–18 days). Vindesine produced no significant abnormalities of hepatic or renal function in the course of this trial.

Although the primary purpose of this study was to define dose tolerance, minor evidence of therapeutic effect was observed in the heavily pretreated group of patients with non-Hodgkin's and Hodgkin's lymphomas, testicular tumors, and acute leukemias (tables 5 and 6). The therapeutic responses were expected in the lymphoma and testicular patients, since these are neoplasms in which vinblastine has therapeutic activity. The therapeutic activity in acute leukemias offers some encouragement that

TABLE 5.—Summary of response to single-dose vindesine therapy—phase I study

Diagnosis	No. of patients: Total	No. of patients: Adequate treatment (%)	Response category: Partial	Minor	No change	Progression
Acute leukemia	4	4 (4)*	0	2	0	2
Non-Hodgkin's lymphoma	7	7 (7)	0	1	3	3
Hodgkin's disease	5	5 (5)	1	1	1	2
Hypernephroma	6	5 (2)	0	1	1	3
Melanoma	3	2 (1)	0	1	0	1
Breast carcinoma	6	6 (0)	0	0	2	4
Colon carcinoma	5	5 (2)	0	0	3	2
Miscellaneous tumors†	11	11 (3)	0	0	2	9
Totals	47	45	1	6	12	26

* Prior vinca alkaloids.

† Soft part sarcoma (2); carcinomas of the head and neck (1), thyroid (1), lung (4), stomach (1); unknown primary (2).

TABLE 6.—Summary of response to daily vindesine therapy—phase I study

Diagnosis	No. of patients: Total	No. of patients: Adequate	Response category: Partial	Minor	No change	Progression
Acute lymphocytic leukemia	7	7	1	3	1	2
Chronic myelogenous leukemia	1	1	0	1	0	0
Non-Hodgkin's lymphoma	3	3	0	2	0	1
Hodgkin's disease	2	2	0	2	0	0
Sarcoma	1	1	0	0	0	1
Head and neck carcinoma	1	0	—	—	—	—
Lung carcinoma	2	2	0	1	1	0
Breast carcinoma	1	1	0	1	0	0
Ovarian carcinoma	1	1	0	0	0	1
Testicular carcinoma	6	6	1	1	0	4
Totals	25	24	2	11	2	9

this vinblastine analog may have more activity in these disorders than was obtained with the parent compound.

Disease-oriented phase II trials have been initiated at this Center using a dose of 4 mg/m² every 7–14 days for good-risk patients ≤ 60 years old with normal liver and marrow functions and a dose of 3 mg/m² for marginal-risk patients (5,6). Daily therapy of 1.3–2.0 mg/m² × 5–7 days at 3-week intervals is appropriate in rapidly proliferating neoplasms such as leukemia, stage III–IV diffuse histiocytic lymphoma, and disseminated testicular cancer. Disease-oriented phase II studies with vindesine are currently in progress at this Center in several signal tumor varieties.

REFERENCES

1. TODD GC, GIBSON WR, and MORTON DM. Toxicology of vindesine (desacetyl vinblastine amide) in mice, rats, and dogs. J Toxicol Environ Health 1:843–850, 1976.

2. DYKE RW, and NELSON RL. Phase I anti-cancer agents. Vindesine (desacetyl vinblastine amide sulfate). Cancer Treat Rev 4:135–142, 1977.

3. CURRIE V, WONG P, TAN R, ET AL. Preliminary clinical studies of desacetyl vinblastine amide sulfate (DVA), a new vinca alkaloid. Proc Am Assoc Cancer Res and ASCO 17:174, 1976.

4. BODEY GP, and FREIREICH EJ. Initial clinical studies of vindesine (desacetyl vinblastine amide sulfate). Proc Am Assoc Cancer Res and ASCO 17:128, 1976.

5. WONG PP, YAGODA A, CURRIE VE, ET AL. Phase II study of vindesine sulfate in the therapy for advanced renal carcinoma. Cancer Treat Rep 61:1727–1729, 1977.

6. YOUNG C, CURRIE V, CVITKOVIC E, ET AL. Early phase II evaluation of vindesine in patients with advanced cancer (Proceedings of the 10th International Congress of Chemotherapy, Zurich, 1977). *In* Current Chemotherapy (Siegenthaler W, and Luthy R, eds). Washington, American Society of Microbiology, 1978, vol 2, pp 1330–1331.

Chronochemotherapy of L1210 Leukemic Mice With Cytosine Arabinoside or Cyclophosphamide [1,2]

William C. Rose,* Mary W. Trader, W. Russell Laster, Jr, and Frank M. Schabel, Jr [3]

SUMMARY

The ability to withstand greater doses of either cytosine arabinoside (ara-C) or cyclophosphamide (CPA) and the effect such higher doses of these drugs have upon L1210 leukemia were evaluated in mice adapted to alternating 12-hour cycles of light (6 AM to 6 PM) and dark. Ara-C was administered in increasing and decreasing ("sinusoidal") doses q3h × 8 once (single course) or q4d × 2 (two courses), such that most (81%) of the total dose was given during the hours from 8 AM to 5 PM. The cumulative maximum tolerated dose of ara-C was 50%–100% greater when given sinusoidally than when given in a conventional, equal-dose manner (same dose at 3-hour intervals). Despite the use of elevated levels of ara-C given sinusoidally to mice bearing L1210 leukemia, no greater therapeutic effect was realized than was obtained among leukemic mice receiving an equitoxic (albeit lower) total dose of ara-C administered in a conventional manner. We also found that the incidences of resistance to ara-C among L1210 populations obtained from relapsing leukemic mice treated previously with the drug were very similar, regardless of the total amount of ara-C given to the leukemic mice or the manner (conventional or sinusoidal) of drug administration. The single-dose LD10 of CPA was 324 mg/kg to 365 mg/kg depending upon which of eight selected times around the clock (3-hour intervals beginning at 8 AM) the injection was made. Marginally higher (approximately 10%) LD10 values were obtained when treatment was given between 8 PM and 5 AM (during the dark cycle). However, no consistent therapeutic advantage was associated with any particular single time or period of time (eg, 8 PM to 5 AM) of injection of CPA for mice bearing L1210 leukemia.

[Cancer Treat Rep 62:1337–1349, 1978]

Biologic rhythms of many different frequencies are an inherent characteristic of animal life. One frequency that has achieved a great amount of attention is that which corresponds to the 24-hour rotation of the earth, referred to as "circadian" (1). Circadian fluctuations of many physiologic parameters have been studied (2,3). For example, circadian variation has been reported in the labeling index for rat corneal epithelium (4) and the level of tritiated thymidine incorporated into DNA of mouse thymus (5), spleen (6), bone marrow, and duodenum (7). With regard to these last two tissues, the phasing of the rhythms for DNA synthesis was very similar in mice standardized to 12 hours of light from 6 AM to 6 PM alternating with 12 hours of darkness, the least amount of DNA synthesis occurring during the dark cycle. Because of these rhythmic fluctuations, an organism may respond differently to the same stimulus at different times and chronotherapy attempts to take into account the times of maximal resistance to the undesired effects of treatment.

Knowledge of when DNA synthesis is lowest in bone marrow and gut epithelium could presumably be exploited in treating mice with cell-cycle specific agents which inhibit DNA synthesis and for which these tissues represent primary targets. However,

[1]Received Mar 27, 1978; revised July 5, 1978; accepted July 14, 1978.

[2]Supported by contract N01–CM–43756 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Chemotherapy Research Department, Southern Research Institute, Birmingham, Ala.

*Reprint requests to: Dr. William C. Rose, Chemotherapy Research Department, Southern Research Institute, 2000 9th Ave S, Birmingham, Ala 35205.

Scheving et al (7) reported that the peak in mortality occurring in standardized (light cycle, 6 AM to 6 PM) mice receiving cytosine arabinoside (ara-C), an inhibitor of DNA synthesis, was associated with administration of the drug during that phase of the circadian cycle when DNA synthesis in mouse duodenum and bone marrow was lowest. Nevertheless, attempts continue to be made, as in the studies reported here, to use chronopharmacologic concepts to optimize chemotherapy.

Circadian susceptibility cycles to agents used in cancer chemotherapy have been reported for adriamycin (8), cyclophosphamide (CPA) (9), ara-C (2,10,11), and CPA in combination with ara-C (12). For example, based on limited data, of standardized (light cycle, 6 AM to 6 PM) mice receiving doses of 10.4 mg/kg of adriamycin ip on Day 1 and 20.8 mg/kg of adriamycin ip on Day 5, the number surviving 2 weeks after treatment was greatest (three of six mice) when both drug injections were given at 10 PM compared to all other times of injection evaluated (one of 30 mice); by 4 weeks after treatment, none of the groups had more than one of six mice alive (8). With CPA, a single ip dose of 375 mg/kg caused less mortality in standardized (light cycle, 6 AM to 6 PM) mice given the drug at 4 PM or 8 PM (eg, LD25) compared to other times (eg, LD70) during the circadian cycle (9).

Our interest in chronochemotherapy stems from reports by others involving the treatment of mice bearing L1210 leukemia with ara-C (2,10), or ara-C plus CPA (12), according to chronobiologic principles as compared to more conventional methods. For example, Haus et al (2,10) reported that leukemic mice survived significantly longer when ara-C was given, not in the conventional manner of equal doses at 3-hour intervals (ie, q3h × 8, q4d × 4) as used by Skipper et al (13), but in "sinusoidally" (10) increasing and decreasing doses during each 24-hour course of treatment, the largest amounts being given at the previously determined times of peak host resistance to the drug (from 8 AM to 5 PM, for mice adapted to light daily from 6 AM to 6 PM alternating with darkness). Because of our long interest and involvement with optimal scheduling of anticancer agents in the treatment of murine leukemias, we attempted to demonstrate the reported assets of chronochemotherapy using either ara-C or CPA to treat mice bearing L1210 leukemia.

MATERIALS AND METHODS

Male BDF_1 (C57BL/6 × DBA/2) or CDF_1 (BALB/c × DBA/2) mice, 6–8 weeks old, were caged individually and held in a room at 23°C ± 1°C with artificial illumination daily from 6 AM to 6 PM (natural lighting was excluded at all times). All entries into this room were restricted, and scheduled duties were performed with a minimum of disturbance. Wayne Lab Blox^R (Allied Mills, Chicago, Ill) and water were provided ad libitum. Except where specified (two groups of mice were included for comparison in one experiment), all mice were subjected to these conditions for 8–11 days prior to leukemic cell implant or 9–13 days prior to receiving an initial drug injection and were maintained in this manner until the experiments were terminated. We considered these experimental conditions to be essentially similar to those used by others reporting therapeutic improvement using chronochemotherapy (2,10,12).

Mice not maintained under the conditions just described are referred to as kept "without standardization," or similar terminology. The ordinary laboratory conditions that these mice were subjected to included (*a*) being housed ten mice to a cage in a well-lighted laboratory with mice from many other experiments, (*b*) artificial light from 6 AM to 4:30 PM with more sunlight during the warmer seasons (experiments were conducted over a 2-year period), and (*c*) frequent but unscheduled disturbances, including artificial lighting, throughout each and every 24-hour period as necessitated by the injection, feeding, watering, and cage changing requirements of thousands of mice in other experiments.

BDF_1 and CDF_1 mice, used in drug toxicity studies, were observed for 30–54 days after treatment. CDF_1 mice, used in leukemia experiments, were observed for 40–60 days after leukemic cell implant (34–54 days after treatment).

L1210 leukemia ascites cells, obtained from DBA/2 mice, were implanted ip into CDF_1 mice (10^6 or 10^7 cells/mouse). A $\log_{10}$ serial dilution titration of the donor L1210 cell suspensions used in each experiment was included to allow objective estimates of viable tumor cells surviving drug treatment (14). Treatment of leukemic mice was initiated 46 hours after implant. Nonleukemic CDF_1 drug toxicity control mice were included in each leukemia experiment and were treated in parallel with the leukemic mice.

All of the drugs used in this study were obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. Saline solutions of ara-C and CPA were given ip on an exact body weight basis (0.01 ml/g). Injections from 6 PM to 6 AM were made under conditions of dim lighting and were performed with a minimal amount of disturbance. One or two courses of ara-C were administered on a q3h × 8 basis, with a q4d × 2 schedule used for the two-course treatments. The total daily amounts of ara-C were administered in either eight equal

doses ("homeostatic" [11,12], which is the conventional schedule) or, in different mice, eight sinusoidal doses. The particular sinusoidal dosing schedule used has been described as being effective in permitting mice to tolerate greater total amounts of ara-C (2,10,11). This schedule consists, briefly, of giving the major portion of any total daily ara-C dose during the hours from 8 AM to 5 PM, when the mice are the least susceptible to the toxic effects of the drug. CPA was always administered as a single dose at one of eight selected times during the circadian cycle. The length of time required to inject all of the mice at a single treatment period (ara-C or CPA) never exceeded 75 minutes, with the direct involvement of each mouse usually limited to < 30 seconds. The same sequence of drug treatment of multiple groups at each time period was always followed so that the interval between multiple doses was constant.

The relative effectiveness of the various treatment regimens was judged on the basis of survival rates at the end of an experiment and the median survival time (MST) of *dying mice only*. Percent increase in lifespan (ILS) was calculated by dividing the MST of treated mice by the MST of the untreated leukemic control mice, subtracting 1, and multiplying by 100. The approximate number of L1210 cells surviving at the end of drug treatment was estimated from the lifespans of the untreated leukemic control mice and the drug-treated mice that died, the time of last treatment, and the calculated doubling time of the leukemia cells (14).

To determine if leukemic cell populations present in mice following treatment with ara-C contained cells resistant to ara-C, 12 relapsing mice were killed (on the median day of death as determined in parallel groups of mice receiving the identical drug therapy) and 10^5 (10^6 on one occasion) leukemic ascites cells were transplanted into each of 20 CDF_1 recipients per donor. On Day 2 after implant, ten of these 20 mice were administered a dose of 125 mg/kg of palmo-ara-C ip, and the remaining ten were left untreated to serve as parallel controls. Resistance to ara-C was judged by the ability of palmo-ara-C to delay deaths due to leukemia relative to the mortality rate among the parallel, untreated, leukemic control mice, and relative to similarly treated bioassay recipients of leukemic cells obtained from non-drug-treated leukemic donors.

LD10 values were determined by probit analysis (15) in the CPA toxicity experiments and statistical comparisons were made using analysis of variance. The maximum tolerated dose (MTD) was defined as the dose which was lethal to ≤ 10% of the drug-treated mice.

RESULTS

Ara-C Toxicity in BDF_1 Mice

We wished to determine if sinusoidal dosing would permit mice to tolerate more ara-C than if they had been given equal doses of the drug throughout a q3h × 8 treatment schedule (table 1). When given as equal doses (groups 1–4), the MTD for ara-C, based on two experiments, was 160 mg/kg/day. In comparison, all of the mice in group 6 receiving sinusoidal doses of ara-C survived a total exposure of 240 mg/kg (MTD). This 50% increase in tolerated drug dosage was achieved using a sinusoidal schedule in which the initial injection was made at 8 AM and 81% of the total ara-C dose was given during the first four injections (from 8 AM to 5 PM).

None of the other sinusoidal schedules (groups 9–11, table 1) was as advantageous as that (group 6) just described. The time of initiation of drug ther-

TABLE 1.—Lethal toxicity of ara-C given every 3 hrs in 8 sinusoidally increasing and decreasing doses as compared to 8 equal doses in BDF_1 mice adapted to alternating 12-hr cycles of light and dark

	Ara-C dose (mg/kg ip) given at—												Total dose (mg/kg)	30-day survivors/ total treated
Group*	8 AM	11 AM	2 PM	5 PM	8 PM	11 PM	2 AM	5 AM	8 AM	11 AM	2 PM	5 PM		
1C	35	35	35	35	35	35	35	35					280	10/20
2C	30	30	30	30	30	30	30	30					240	11/20
3C	25	25	25	25	25	25	25	25					200	11/20
4C	20	20	20	20	20	20	20	20					160†	18/20
5S	35	78.8	78.8	35	17.5	8.8	8.8	17.5					280	13/20
6S	30	67.5	67.5	30	15	7.5	7.5	15					240†	20/20
7S	25	56.3	56.3	25	12.5	6.3	6.3	12.5					200	19/20
8S	20	45	45	20	10	5	5	10					160	20/20
9S	15	7.5	7.5	15	30	67.5	67.5	30					240	6/10
10S					30	67.5	67.5	30	15	7.5	7.5	15	240	15/20
11S					15	7.5	7.5	15	30	67.5	67.5	30	240	13/20

*C = conventional dosing (equal doses); S = sinusoidal dosing.
†The MTD based upon the combined results of 2 experiments.

TABLE 2.—Response of L1210 leukemia to ara-C given on both equal- and sinusoidal-dose schedules in CDF_1 mice adapted to alternating 12-hr cycles of light and dark (experiment 1, Apr 1976)

Treatment: Ara-C treatment of mice implanted ip with 10^6 L1210 cells: mg/kg ip given at—								Schedule (days after implant)	Total dose (mg/kg)	40-day survivors/ total treated	MST (dying mice only)	%ILS	Approximate No. of tumor cells alive at end of therapy*	Lethal drug toxicity in nonleukemic mice (No. of survivors/ total treated)
8 AM	11 AM	2 PM	5 PM	8 PM	11 PM	2 AM	5 AM							
Saline	→							2	—	0/10	8.0	—		
Saline	→							2,6	—	0/10	8.0	—		
35	35	35	35	35	35	35	35	2	280	0/10	14.0	75		7/10
30	30	30	30	30	30	30	30	2	240	0/10	15.0	87		8/10
25	25	25	25	25	25	25	25	2	200	0/10	15.0	87†	1.4×10^2	10/10
20	20	20	20	20	20	20	20	2	160	0/10	15.0	87	1.4×10^2	10/10
40	90	90	40	20	10	10	20	2	320	0/10	15.0	87†	1.4×10^2	9/10
35	78.8	78.8	35	17.5	8.8	8.8	17.5	2	280	0/9	14.0	75	1.3×10^3	10/10
30	67.5	67.5	30	15	7.5	7.5	15	2	240	0/10	15.0	87	1.4×10^2	10/10
30	30	30	30	30	30	30	30	2,6	480	0/10	10.0	25		0/10
25	25	25	25	25	25	25	25	2,6	400	0/10	21.5	168		0/10
20	20	20	20	20	20	20	20	2,6	320	0/10	22.0	175		8/10
15	15	15	15	15	15	15	15	2,6	240	1/10	22.0	175†	About 1(2.3)	10/10
35	78.8	78.8	35	17.5	8.8	8.8	17.5	2,6	560	0/10	22.0	175		3/10
30	67.5	67.5	30	15	7.5	7.5	15	2,6	480	0/10	22.0	175		4/10
25	56.3	56.3	25	12.5	6.3	6.3	12.5	2,6	400	1/10	22.0	175†	About 1(2.3)	10/10

*Based on median day of death of dying mice. Nos. in parentheses are based upon % survivors (14).
†Obtained at the MTD in concurrently treated nonleukemic toxicity control mice.

apy and size of the initial doses appeared to be critical variables in determining the survival rate. Administering the higher doses of ara-C during the dark cycle (from 8 PM to 5 AM) resulted in 25%–40% mortality at the 240-mg/kg/day dose level, regardless of whether treatment was initiated at 8 AM (group 9) or 8 PM (group 10). Administering the greater portion (81%) of 240 mg/kg/day of ara-C during the light cycle (from 8 AM to 5 PM), but initiating treatment during the dark cycle (group 11), likewise resulted in excessive mortality (35%).

Ara-C Toxicity in CDF_1 Mice

CDF_1 drug toxicity control mice were included in all leukemia studies. As had been observed in BDF_1 mice, CDF_1 mice maintained on 12-hour light-dark cycles and receiving sinusoidal doses of ara-C on a q3h × 8 single-course schedule tolerated more drug than mice receiving equal doses of ara-C. Enhanced drug tolerance for mice receiving ara-C sinusoidally was also seen when two courses of ara-C were given 4 days apart (q3h × 8, q4d × 2).

Three experiments were conducted; the first was initiated in April 1976, the second in August 1976, and the third in April 1977. In the first experiment (table 2), the MTD for a single course of ara-C given sinusoidally was 320 mg/kg; when given as equal doses the MTD was 200 mg/kg. When two courses of ara-C were given, the cumulative MTD in mice receiving sinusoidal doses was 400 mg/kg, compared to 240 mg/kg in mice treated on a homeostatic schedule. Thus, by using sinusoidal dosing, the MTD for ara-C was increased about 60%.

In the second experiment (table 3), sinusoidal dosing again increased the total MTD of ara-C. A single course of 160 mg/kg of ara-C given on a homeostatic treatment schedule was lethal for 20% of the mice, but the MTD of ara-C given sinusoidally was 240 mg/kg. On a q3h × 8, q4d × 2 schedule, the MTD was 240 mg/kg on the homeostatic schedule and 480 mg/kg on the sinusoidal schedule. In the latter instance, twice as much drug was tolerated on the sinusoidal schedule compared to the homeostatic regimen.

In the third experiment (table 4), mice were treated with two courses of ara-C only. For those mice treated with a sinusoidal regimen, the MTD was a total dose of 520 mg/kg. Mice that received ara-C on a homeostatic schedule tolerated only 280 mg/kg; a 320-mg/kg dose killed 20% of the mice. This difference represented a > 60% increase in the MTD for ara-C, which was consistent with the other experimental results (tables 1–3).

TABLE 3.—Response of L1210 leukemia to ara-C given on both equal- and sinusoidal-dose schedules in CDF_1 mice adapted to alternating 12-hr cycles of light and dark (experiment 2, Aug 1976)

Treatment													
Ara-C treatment of mice implanted ip with 10^6 L1210 cells: mg/kg ip given at—								Schedule (days after implant)	Total dose (mg/kg)	45-day survivors/ total treated	MST (dying mice only)	%ILS	Approximate No. of tumor cells alive at end of therapy*	Lethal drug toxicity in nonleukemic mice (No. of survivors/ total treated)
8 AM	11 AM	2 PM	5 PM	8 PM	11 PM	2 AM	5 AM							
No therapy →								—	—	0/20	7.0	—		10/10
40	40	40	40	40	40	40	40	2	320	0/10	6.5	–8		5/10
30	30	30	30	30	30	30	30	2	240	0/10	14.5	107		7/10
20	20	20	20	20	20	20	20	2	160	0/10	14.0	100	4.0×10^8	8/10
50	112.5	112.5	50	25	12.5	12.5	25	2	400	0/10	14.0	100		7/10
40	90	90	40	20	10	10	20	2	320	0/10	14.0	100		7/10
30	67.5	67.5	30	15	7.5	7.5	15	2	240	0/10	14.0	100†	4.0×10^8	8/8
30	30	30	30	30	30	30	30	2,6	480	0/10	9.0	28		0/10
25	25	25	25	25	25	25	25	2,6	400	0/10	10.0	42		2/10
20	20	20	20	20	20	20	20	2,6	320	1/10	20.0	185		6/10
15	15	15	15	15	15	15	15	2,6	240	1/10	20.0	185†	8(2.3)	10/10
35	78.8	78.8	35	17.5	8.8	8.8	17.5	2,6	560	5/10	10.0	42		3/10
30	67.5	67.5	30	15	7.5	7.5	15	2,6	480	1/10	20.0	185†	8(2.3)	9/10
25	56.3	56.3	25	12.5	6.3	6.3	12.5	2,6	400	0/10	20.0	185	8	10/10

*Based on median day of death of dying mice. Nos. in parentheses are based upon % survivors (14).
†Obtained at the MTD in concurrently treated nonleukemic toxicity control mice.

TABLE 4.—Response of L1210 leukemia to ara-C given on both equal- and sinusoidal-dose schedules in CDF_1 mice adapted to alternating 12-hr cycles of light and dark (experiment 3, Apr 1977)

Ara-C treatment of mice implanted ip with 10^6 L1210 cells: mg/kg ip given on Days 2 and 6 after implant at—								Total dose (mg/kg)	60-day survivors/ total treated	MST (dying mice only)	%ILS	Approximate No. of tumor cells alive at end of therapy*	Lethal drug toxicity in nonleukemic mice (No. of survivors/ total treated)
8 AM	11 AM	2 PM	5 PM	8 PM	11 PM	2 AM	5 AM						
Saline →								—	0/10	7.0	—		
25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	400	0/10	19.5	178		4/10
22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	360	0/10	21.0	200		7/10
20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	320	0/10	22.0	214†	About 1	8/10
17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5	280	0/8	21.0	200	About 1	10/10
15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	240	0/8	20.0	185	4	10/10
37.5	84.3	84.3	37.5	18.8	9.4	9.4	18.8	600	1/10	21.0	200		3/10
35.0	78.8	78.8	35.0	17.5	8.8	8.8	17.5	560	0/10	20.0	185		6/10
32.5	73.1	73.1	32.5	16.3	8.2	8.2	16.3	520	1/10	21.0	200	About 1(2.3)	10/10
30.0	67.5	67.5	30.0	15.0	7.5	7.5	15.0	480	0/10	20.5	192†	About 1	10/10
27.5	61.9	61.9	27.5	13.8	6.9	6.9	13.8	440	1/9	19.5	178	11(2.3)	9/10
25.0	56.3	56.3	25.0	12.5	6.3	6.3	12.5	400	2/9	21.0	200	About 1(1.6)	Not determined

*Based on median day of death of dying mice. Nos. in parentheses are based upon % survivors (14).
†Obtained at a dose that was not lethal in leukemic mice.

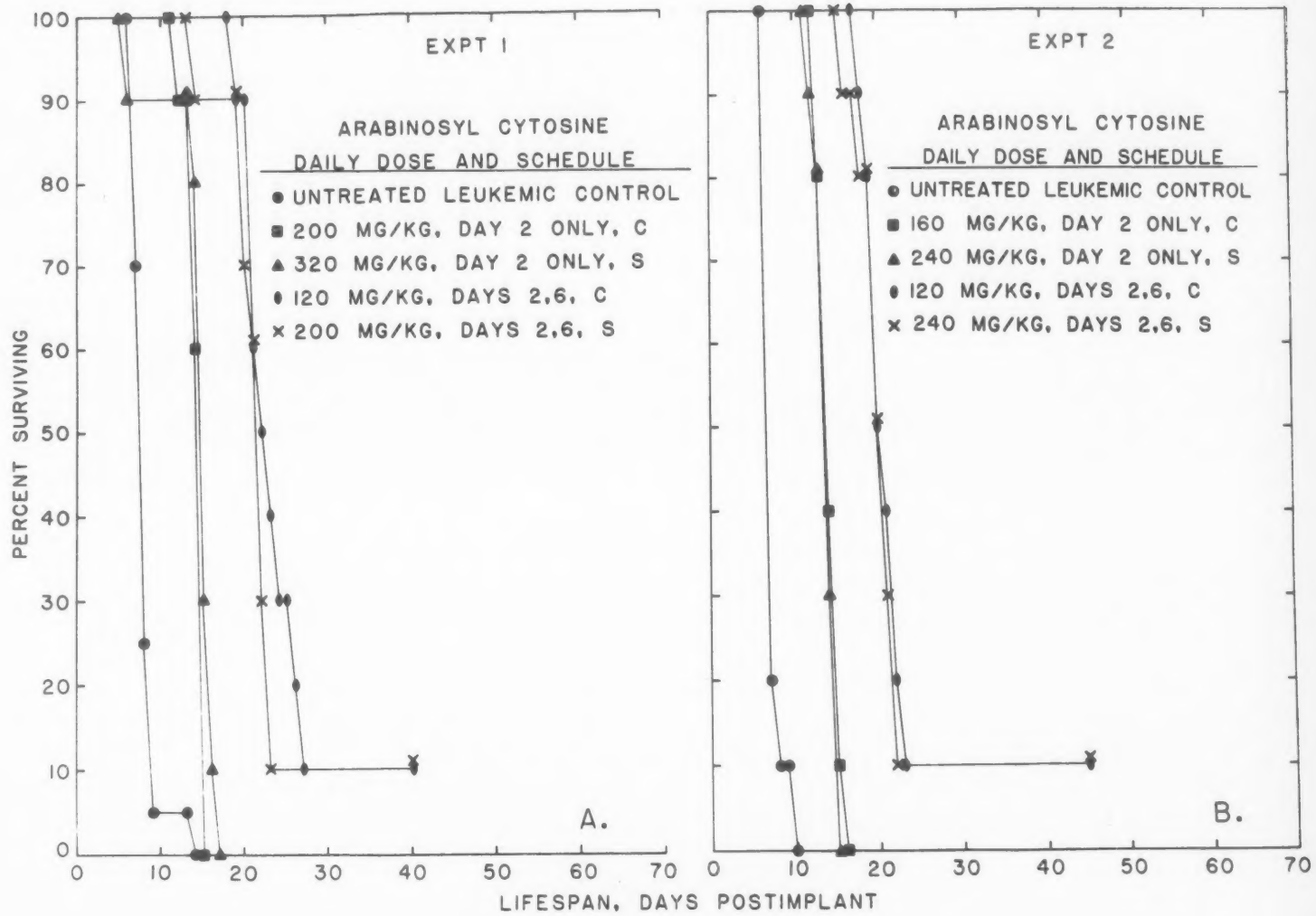

FIGURE 1.—Optimal responses of CDF_1 mice implanted ip with 10^6 L1210 cells and treated with ara-C at the MTD either sinusoidally (S) or conventionally (C) q3h × 8 on Day 2 or Days 2 and 6 after implant. Detailed descriptions of each drug regimen are given in tables 2 (experiment 1) and 3 (experiment 2). All mice (10 mice/group except for untreated leukemic controls, 20 mice/group) were exposed to alternating 12-hr cycles of light (6 AM to 6 PM) and dark.

Ara-C Treatment of L1210 Leukemia

In an attempt to take advantage of their increased tolerance for ara-C, mice maintained on 12-hour light-dark cycles were implanted ip with 10^6 L1210 leukemia cells and treated with one or two courses of sinusoidal doses of ara-C (q3h × 8 on Day 2, or Days 2 and 6, after implant). Leukemic mice receiving ara-C on a homeostatic treatment schedule were treated concurrently. Despite the additional drug received and tolerated by mice treated in a sinusoidal fashion, no therapeutic advantage was seen.

In the first experiment (table 2 and fig 1A), among conventionally dosed leukemic mice given one course of ara-C totaling 200 mg/kg (MTD), the MST was 15 days, which represented an 87% ILS compared to that of saline-treated leukemic control mice. Leukemic mice receiving as much as 320 mg/kg of ara-C (MTD), given as sinusoidal doses in one course, had the same MST and hence the same %ILS as the conventionally treated mice receiving only 63% as much drug. Similar therapeutic responses were also observed among conventionally and sinusoidally dosed leukemic mice given two courses of ara-C. At MTD levels of ara-C (240 mg/kg homeostatic and 400 mg/kg sinusoidal), both groups of leukemic mice had a 10% survival rate and an MST of 22 days among the dying mice (175% ILS). Thus, the optimum responses of leukemic mice receiving one or two courses of ara-C, at equitoxic doses by either treatment approach, were identical.

Additionally, we titrated an L1210 cell suspension in $\log_{10}$ dilutions down to "one" cell in CDF_1 mice that had been standardized to 12-hour alternating light (6 AM to 6 PM) and dark cycles and also in parallel groups of mice maintained without standardization. Tumor cell population doubling times calculated (14) from lifespan and survival data from these titrations (not shown) were 0.31 day in the standardized mice and 0.35 day in nonstandardized mice. These comparative values indicate that the growth kinetics of the tumor cell population were not significantly altered by standardization of the host mice.

Results from the second experiment are presented separately in table 3 and figure 1B to document the high degree of reproducibility between experiments conducted 4 months apart. Once again, the optimum responses obtained at comparable levels of drug toxicity were equivalent among groups of leukemic mice, regardless of whether equal or sinusoidal doses of ara-C were administered.

Administration of a single course of ara-C on Day 2 after implant (q3h × 8) resulted in a 100% ILS among leukemic mice receiving a total dose of 160 mg/kg (lethal to 20% of the mice) on a homeostatic schedule or 240 mg/kg (MTD) on a sinusoidal schedule. This compared well with the optimum response of 87% ILS for a single course of therapy seen in the first experiment (table 2 and fig 1A). When two courses of ara-C were employed (q3h × 8, Days 2 and 6 after implant), the optimum responses of leukemic mice treated by either dosing method were identical, 185% ILS and one of ten mice cured. This was accomplished at a total dose of 240 mg/kg (MTD) on the homeostatic schedule and 480 mg/kg (MTD) on the sinusoidal schedule. Thus, despite the leukemic mice having tolerated twice as much drug, no increase in therapeutic activity of ara-C was obtained with sinusoidal dosing. Note that the best therapeutic responses achieved with two courses of ara-C (using either sinusoidal or homeostatic dosing) were similar in both experiments (175% ILS, one of ten mice cured, versus 185% ILS, one of ten mice cured). It should also be pointed out that, although two sinusoidal courses of ara-C at a total dose of 560 mg/kg did result in cures in five of ten mice (table 3), this was accomplished only with unacceptable toxicity (lethal for seven of ten non-tumor-bearing control mice and, judging from their death pattern, five of ten leukemic mice).

The third experiment (table 4) differed from the previous two in that more dose levels, with smaller increments between levels, were evaluated, and relapsing mice were bioassayed for the presence of leukemic cells resistant to ara-C. In addition, ara-C was given q3h × 8 on Days 2 and 6 after implant only (a single course of therapy was not used).

The optimal therapeutic response using homeostatic dosing was achieved with a total dose of 320 mg/kg of ara-C which gave a 214% ILS without any cures. Although this dose was lethal for 20% of the parallel, nonleukemic, drug toxicity control mice, it was apparently not lethal to leukemic mice judging from both the mortality pattern (the first death did not occur until 13 days after treatment) and the presence of splenomegaly and ascitic fluid at necropsy. A total dose of only 240 mg/kg also resulted in a near optimal response of 185% ILS. In comparison, among mice receiving ara-C sinusoidally, the best therapeutic response was achieved using a total dose of 400 mg/kg which cured two of nine mice and gave a 200% ILS among the dying mice. At higher doses, ≤ 480 mg/kg of ara-C (MTD), no improvement in therapeutic response was observed. In terms of how many leukemic cells were estimated to have survived therapy (14), there was no substantial difference between the optimal effects achieved with homeostatic as compared to sinusoidal dosing,

TABLE 5.—Resistance to ara-C among L1210 leukemia cells obtained from relapsing leukemic mice following various therapies using ara-C

L1210 cells obtained from mice treated with ara-C (mg/kg ip)*			No. of L1210 cells implanted ip into new hosts	Results of treatment with 125 mg/kg of palmo-ara-C on Day 2 after implant			
Total dose	Given conventionally or sinusoidally	Donor No.		60-day survivors/ total treated	MST (dying mice only)	%ILS†	Comments‡
None	Untreated leukemic controls	1	10^6	2/10	26.5	278	Typical patterns of ara-C sensitivity
		2	10^5	6/10	22.5	150	
		3	10^5	5/10	28.0	211	
		4	10^5	3/10	22.0	144	
440	Sinusoidally	1	10^5	0/10	16.0	100	PR
		2	10^5	0/10	11.0	15	CR
		3	10^5	2/10	26.5	278	S
400	Sinusoidally	1	10^5	1/10	32.0	255	S
		2	10^5	4/10	27.0	200	S
		3	10^5	0/10	13.0	62	CR
280	Conventionally	1	10^5	7/10	26.0	188	S
		2	10^5	4/10	42.5	372	S
		3	10^5	4/10	29.5	227	S
240	Conventionally	1	10^6	1/10	26.0	225	S
		2	10^5	4/10	27.0	237	S
		3	10^5	0/10	10.0	–10	CR

*All treatments were q3h × 8, q4d × 2 following ip implantation of 10^6 L1210 cells. The specific doses administered at each injection were the same as described in table 4 for each respectively treated group of mice.

†Compared to parallel groups of untreated controls implanted with the same number of L1210 cells from the same donors.

‡S = sensitive (≥ 1 survivor and/or ≥ 200% ILS); PR = partial resistance (no survivors but ≥ 100% ILS); CR = complete resistance (no survivors and < 100% ILS).

the limited number of long-term survivors obtained with sinusoidal dosing notwithstanding.

Assay for L1210 Cells Resistant to Ara-C

We would have predicted that treatment of mice bearing L1210 populations of ≥ 10^6 cells would be unsuccessful using ara-C because of the presence of cells resistant to the drug (16,17). Increasing the total dose of ara-C up to the MTD should not result in the killing of ara-C-resistant cells. Thus, despite the greater amount of ara-C tolerated by mice adapted to fixed cycles of light and dark, and treated according to sinusoidal dosing procedures, the emergence of ara-C-resistant cells should be found in such mice to the same extent as in leukemic mice treated by conventional dosing methods and at lower total dose levels.

To test this hypothesis, relapsing leukemic mice were killed and their leukemic ascites cells bioassayed for resistance to ara-C. Of three donor mice randomly selected on the median day of death from among a group treated with 240 mg/kg of ara-C on a homeostatic schedule (q3h × 8, q4d × 2), one mouse yielded L1210 cells markedly resistant to the drug (table 5). This incidence (one of three mice) was the same among bioassayed mice treated sinusoidally with as much as 400 or 440 mg/kg of ara-C, thereby supporting our prediction that resistance to ara-C would not be overcome by increasing ara-C dosage to the higher MTD of ara-C obtained with sinusoidal treatment.

CPA Toxicity in CDF_1 Mice

We wished to determine if mice were more tolerant of CPA during a certain portion of each 24-hour period than at other times. Four experiments were performed in which single injections of CPA were given at several dose levels, at one of eight selected times, to nonleukemic mice that had been acclimated to a schedule of alternating 12-hour cycles of light (6 AM to 6 PM) and dark. The doses of CPA and treatment times are shown in table 6.

Based upon the LD10 values calculated for each injection time (table 6 and fig 2), marginally greater tolerance for CPA was found when the drug was given during the dark cycle, 6 PM to 6 AM ($P < 0.005$). At these times, the calculated LD10 values ranged from 352 to 365 mg/kg. In contrast, the calculated LD10 values of CPA given during the light cycle (6 AM to 6 PM) ranged from 324 to 339

TABLE 6.—Toxicity of single doses of CPA given to CDF_1 mice adapted to alternating 12-hr cycles of light and dark

Dose of CPA (mg/kg ip)	Experiment No.	% survivors (10 mice/group) on Day 45 in each of 4 experiments following administration of CPA at different times							
		8 AM	11 AM	2 PM	5 PM	8 PM	11 PM	2 AM	5 AM
550	2	0	0	0	0	0	0	10	0
500	1	0	0	0	0	0	10	20	0
	2	0	0	0	0	40	60	20	20
	3	0	10	0	10	10	20	10	0
	4	50	50	60	80	60	90	80	70
450	2	20	30	0	20	20	60	70	30
	3	30	30	20	20	70	20	30	30
	4	80	60	80	60	70	80	80	90
400	1	30	50	30	70	50	80	60	30
	2	30	80	60	70	90	40	90	80
	3	30	60	70	30	60	50	60	80
	4	100	70	80	90	80	90	80	70
350	2	90	90	90	90	100	100	100	100
	3	100	90	100	90	100	90	60	100
	4	100	100	100	80	80	100	100	90
300	1	80	90	100	90	100	100	90	100
	2	100	90	100	90	90	100	100	100
	3	90	100	80	100	90	100	100	100
LD10 ± SE		324 ± 7	339 ± 7	330 ± 7	334 ± 8	353 ± 8	365 ± 8	363 ± 8	352 ± 8

mg/kg. The greatest difference between the lowest LD10 of CPA (324 mg/kg at 8 AM) and the highest LD10 (365 at 11 PM) was only 41 mg/kg (13%).

CPA Treatment of L1210 Leukemia

In parallel with the last two toxicity experiments involving CPA, mice were implanted ip with 10^7 L1210 cells and treated with single doses of CPA (300, 350, 400, or 450 mg/kg) on Day 2 after implant at the treatment times shown in table 7. All dying mice were necropsied and the presence or absence of overt leukemia (ascitic fluid and/or splenomegaly) was noted. The designation of a given dose as the MTD was based not on the survival of the parallel drug toxicity control mice but rather on the presence or absence of overt leukemia. The results of both experiments are presented in table 7 (optimal results only, fig 3).

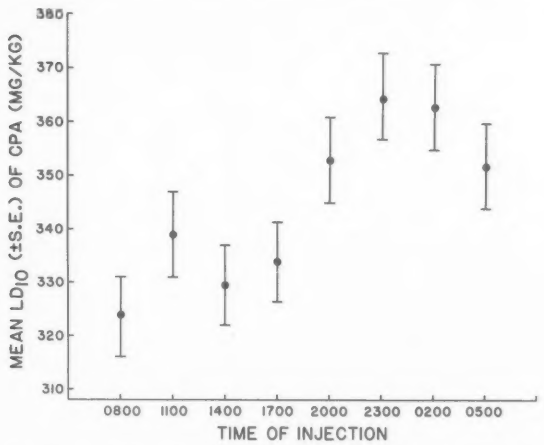

FIGURE 2.—Toxicity of single ip doses of CPA administered at various times to CDF_1 mice. Results are expressed in terms of the mean LD10 and the SE determined from 4 experiments in which the mice (180 mice at each treatment time) were exposed to alternating 12-hr cycles of light (6 AM to 6 PM) and dark.

In the first study, marginal therapeutic advantage, obtained by administering CPA at either 5 PM or 8 PM, might be presumed. At these times, CPA cured (ie, alive on Day 45) two of ten mice and caused a 171% ILS among those dying (350 mg/kg at 5 PM), or cured one of ten mice and achieved a 200% ILS (400 mg/kg at 8 PM). Although CPA at other times of injection failed to cure any mice, even the lowest ILS observed (114%) indicated that the number of viable L1210 cells surviving therapy was about ten cells per mouse (14).

In the second experiment (table 7 and fig 3), an optimal single time or timespan during the circadian cycle for administering CPA was not apparent. Cure rates of 10%–20% were observed following administration of CPA at six of the eight time periods, and the ILS among *all* treated groups reflected about one leukemia cell per mouse surviving therapy (14). Interestingly, in three of the four instances that two of ten mice were cured, the dose

TABLE 7.—Response of L1210 leukemia to single doses of CPA given to CDF_1 mice*

CPA treatment of mice implanted ip with 10^7 L1210 cells: mg/kg ip given on Day 2 after implant								Experiment 1				Experiment 2			
8 AM	11 AM	2 PM	5 PM	8 PM	11 PM	2 AM	5 AM	45-day survivors/ total treated	MST (dying mice only)	%ILS	Approximate No. of tumor cells alive at end of therapy†	45-day survivors/ total treated	MST (dying mice only)	%ILS	Approximate No. of tumor cells alive at end of therapy†
Saline →								0/20	7.0	—	—	0/20	6.0	—	—
450								0/10	13.5	92		ND§	ND	ND	ND
400								0/10	18.0	157		1/10	21.0	250‡	About 1(2.3)
350								0/10	18.0	157‡	About 1	1/10	16.0	166	About 1(2.3)
300								0/10	16.5	135	About 1	2/10	17.5	191	About 1(1.6)
	450							0/10	8.5	21		ND	ND	ND	ND
	400							0/10	15.5	121		1/10	21.0	250‡	About 1(2.3)
	350							0/10	16.0	128		0/10	21.5	258	About 1
	300							0/10	15.0	114‡	13	0/10	17.0	183	About 1
		450						0/10	9.0	28		ND	ND	ND	ND
		400						0/10	17.0	142		0/10	19.5	225	
		350						0/10	17.0	142‡	About 1	0/10	19.0	216‡	About 1
		300						0/10	15.0	114	13	0/10	18.0	200	About 1
			450					0/10	15.5	121		ND	ND	ND	ND
			400					0/10	16.0	128		0/10	24.5	308	
			400*					ND	ND	ND	ND	0/10	23.0	283‡	About 1
			350					2/10	19.0	171‡	About 1(1.6)	2/10	18.0	200‡	About 1(1.6)
			350*					ND	ND	ND	ND	0/9	22.0	266	About 1
			300					1/10	18.0	157	About 1(2.3)	0/10	16.5	175	About 1
				450				0/10	18.0	157		ND	ND	ND	ND
				400				1/10	21.0	200‡	About 1(2.3)	0/10	20.5	241‡	About 1
				400*				ND	ND	ND	ND	0/9	26.0	333	
				350				0/10	18.0	157	About 1	0/10	16.0	166	About 1
				350*				ND	ND	ND	ND	1/10	18.0	200‡	About 1(2.3)
				300				0/10	16.0	128	About 1	0/10	16.0	166	About 1
					450			0/10	25.5	264		ND	ND	ND	ND
					400			0/10	27.0	285		0/10	24.0	300‡	About 1
					350			0/10	18.0	157‡	About 1	0/10	19.0	216	About 1
					300			0/10	16.5	135	About 1	2/10	16.0	166	About 1(1.6)
						450		0/10	16.5	135		ND	ND	ND	ND
						400		0/10	19.0	171		1/10	24.0	300‡	About 1(2.3)
						350		0/10	17.0	142‡	About 1	0/10	18.0	200	About 1
						300		0/10	17.0	142	About 1	0/10	16.0	166	About 1
							450	0/10	15.0	114		ND	ND	ND	ND
							400	0/10	18.0	157‡	About 1	1/10	22.0	266‡	About 1(2.3)
							350	0/10	16.5	135	About 1	1/10	18.0	200	About 1(2.3)
							300	0/10	16.0	128	About 1	2/10	16.5	175	About 1(1.6)

*All mice in these experiments, except for those groups marked with an *, were subjected to alternating 12-hr cycles of light (6 AM to 6 PM) and dark in a room maintained at 23°C ± 1°C for 11 or 13 days prior to leukemic cell implant and were held under these conditions for the duration of the experiments. The other mice were kept under ordinary laboratory conditions with no attempt made to regulate temperature, light, noise, etc.

†Based on median day of death of dying mice. Nos. in parentheses are based upon % survivors (14).

‡Obtained at the MTD as determined by necropsy of all dying mice and noting the presence or absence of overt leukemia (ie, splenomegaly and/or ascites fluid).

§ND = not determined.

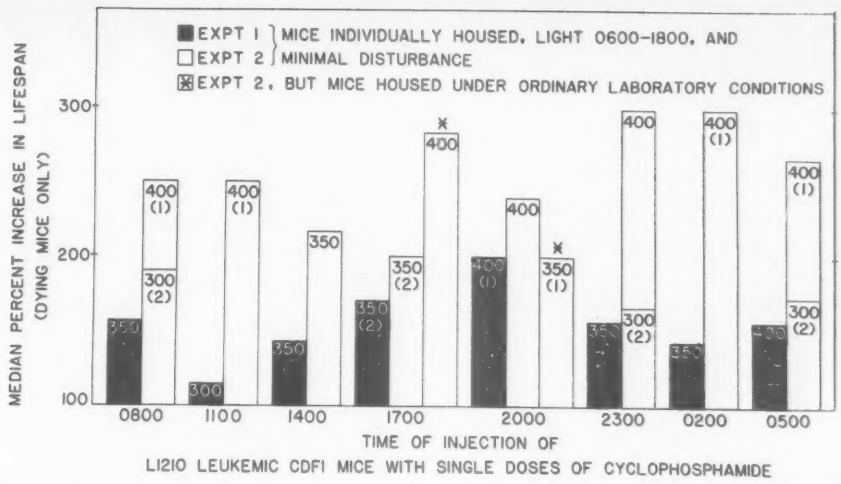

FIGURE 3.—Optimal responses of CDF_1 mice implanted ip with 10^7 L1210 cells and treated with single doses of CPA (MTD), at one of 8 selected times, on Day 2 after implant. Within each bar, doses are shown in mg/kg and the No. of mice alive on Day 45, if any, is shown in parentheses (10 mice/group). The %ILS, compared to untreated leukemic mice with an MST of 7 (experiment 1) or 6 (experiment 2) days, and survival rates in every instance indicated that the viable L1210 cell population remaining at the end of treatment was very small, probably ≤ 10 cells/mouse (14).

of CPA used to effect these responses was less than the MTD for these particular time periods, and in all four instances, the dose used to cure two of ten mice was never > 350 mg/kg. At three of the eight injection times, the dose of CPA that caused the greatest ILS among the dying mice was not the same as that which caused the most cures. For example, 400 mg/kg of CPA given at 11 PM yielded an ILS of 300% (which represented as great an ILS as was observed at any injection time), but 300 mg/kg given at the same time cured two of ten mice while producing only a 166% ILS in dying mice. This is consistent with the marked and commonly seen variation in ILS of dying mice and survival rates with L1210 when viable tumor cell populations are reduced to about one cell per mouse by effective drug treatment at the MTD.

Included in this last experiment were mice maintained under ordinary laboratory conditions (ten mice per cage) with no attempt made to standardize their exposure to light or to limit external stimuli. These mice were implanted in parallel ip with 10^7 L1210 cells and treated with CPA at either 5 PM or 8 PM on Day 2 after implant in parallel with the above-mentioned (standardized) mice. These two times were selected on the basis of the optimal results of the first experiment. The optimal responses obtained at both treatment times are shown in figure 3. Mice kept under ordinary laboratory conditions fared as well as did their counterparts housed under standardized conditions.

Based upon the results of both experiments involving the treatment of mice bearing L1210 leukemia with CPA, no one single time or timespan for drug administration was consistently superior to all other times for the purpose of enhancing therapeutic response. Except for injections made at 2 PM, CPA given at other times resulted in cures in one or two of ten mice in at least one experiment. If 2 PM was the worst time to inject CPA because no mice were cured, note that administration of CPA merely 3 hours later at 5 PM was the only time in both experiments that two of ten mice were cured (ILS of 171%–200%). Yet 5 PM cannot be considered the single best time, for in at least one of the two experiments, CPA given at several other times resulted in cure in one of ten mice and an ILS of 200%–300%. Thus, an optimal time during the circadian cycle for the administration of CPA was not apparent.

DISCUSSION

Using BDF_1 and CDF_1 mice, we have confirmed published reports (2,10,11) indicating that mice have an increased tolerance for ara-C when the drug is administered according to chronobiologic concepts. These mice, when adapted to alternating 12-hour cycles of light (6 AM to 6 PM) and dark, and given ara-C q3h × 8 sinusoidally so that most of each daily total dose was given during the hours from 8 AM to 5 PM, were able to withstand appreciably

more drug (50%–100%) than were the similarly maintained mice given ara-C in equal doses at each treatment time. However, despite their increased tolerance for ara-C, the optimal therapeutic responses of L1210 leukemic mice treated sinusoidally with the drug at MTD levels were no better than those achieved in leukemic mice receiving equitoxic total doses of ara-C by the conventional method (equal doses at each treatment time).

These data regarding treatment of leukemic mice are not in agreement with the conclusions drawn by Haus et al (2,10). These investigators reported that sinusoidal dosing of ara-C significantly prolonged the lifespan of L1210 leukemic mice and increased their cure rate when compared to leukemic mice treated with the *same* total dose of ara-C in a conventional (equal-dose) manner. The two main differences between their experiments and ours are that we compared, whenever possible, the responses obtained at equitoxic dose levels (MTDs), thereby optimizing each treatment method, and we used only one (q3h × 8) or two (q3h × 8, q4d × 2) courses of therapy. Haus et al (2,10) employed four courses (q3h × 8, q4d × 4) of sinusoidal therapy at an acknowledged overdose (LD37), and then compared the result obtained to that achieved with an even more toxic (LD74) equal-dose schedule of ara-C at the same total dose level. We believe that evaluating therapeutic effectiveness against neoplasms at frankly lethal doses is unrealistic and probably misleading. Rather, dose and schedule comparisons should be made at equitoxic doses (≤ LD10), regardless of whether sinusoidal or conventional dosing is used.

One or two courses of treatment were used in our studies because we felt that any indication of therapeutic advantage due to sinusoidal ara-C dosing would become apparent with that degree of therapy. Skipper et al (13) observed, in mice implanted iv with 10^4 L1210 cells or ip with 10^5 L1210 cells, that the therapeutic responses achieved following treatment with three or four courses of ara-C were comparable to those achieved with two courses of therapy. They also reported a 40%–60% cure rate among mice implanted ip with 10^6 L1210 cells using four courses of 120 mg/kg/day of ara-C (15 mg/kg/dose, q3h × 8, Days 2, 6, 10, and 14 after implant). These responses are superior to those reported by Haus et al (2,10) using sinusoidal dosing, including their use of lethal (LD37) ara-C doses (240 mg/kg/day, Days 2, 6, 10, and 14 after implant).

The choice of ara-C for these studies was based solely upon the reports that it was more effective in treating L1210 leukemia when given in a sinusoidal manner (2,10). Our failure to observe increased therapeutic effectiveness with increasing doses of ara-C might have been predicted, based on two established facts related to treatment of L1210 with ara-C. First, treatment with ≤ LD10 doses will cure most mice bearing $< 10^6$ L1210 cells but often fails to cure mice bearing $> 10^6$ cells due to overgrowth of leukemia cells resistant to ara-C (16,17). Since resistance to ara-C in L1210 cells, as well as in several other tumor lines, is due to severe deficiency in the level of detectable ara-C-activating enzyme (deoxycytidine kinase) in the resistant cells (18), one would not expect to kill these cells by increasing the dose of ara-C. Second, previous reports from our laboratories have indicated that the dose of ara-C which will result in the greatest log kill of L1210 cells in vivo, on a q3h × 8 treatment schedule, is well below the LD10 (19). Similar results have been reported following treatment of L1210 cells in vitro with ara-C in that no greater reduction in viability was achieved by increasing the concentration of ara-C in the medium beyond 3.2 μg/ml (20). This concentration exceeds the theoretic maximum plasma level of ara-C in mice treated with the LD10 using a conventional, q3h × 8 schedule (19).

The data presented herein are consistent with the principles and observations reported previously (16–20), and indicate that no therapeutic gain should be expected by increasing dose levels of ara-C over about 15 mg/kg/dose on a q3h × 8 schedule. In view of the particular circumstances associated with resistance to ara-C and the maximum effective cytotoxic doses of ara-C for drug-sensitive L1210 cells in vivo, one could, in retrospect, consider the use of ara-C as being contraindicated in experiments designed to evaluate the influence of sinusoidal dosing on therapeutic effectiveness in standardized mice bearing L1210.

In attempting to evaluate the possible benefits associated with the application of chronobiologic principles, one probably should use drugs (eg, alkylating agents or DNA binders) where there are direct correlations between the size of the dose (up to and perhaps exceeding the LD10) and therapeutic response. We chose CPA because it is a classic and broadly useful alkylating agent.

We determined first the time of greatest tolerance of mice to the lethal effect of CPA. CPA was slightly less toxic (about 10%) when, as a single dose, it was given during the dark cycle, 6 PM to 6 AM. Haus et al (9) found that in mice 4 PM to 8 PM was the time of maximal tolerance to 375 mg/kg of CPA. This is in fair agreement with our observations.

It is unlikely that a mere 10% increase in drug dose would result in a reproducible and noteworthy therapeutic benefit. Certainly in a clinical situation, where drug regimens seldom approach lethal levels, a slightly greater tolerance for CPA, when adminis-

tered at one time as compared to another, would probably not result in consistent and commonly useful therapeutic improvement. Although our first experiment gave an indication of possible therapeutic advantage following administration of CPA at 5 PM or 8 PM, no one time or timespan was associated with superior therapeutic responses to CPA in the second study. Furthermore, in this latter instance, responses comparable to the best responses achieved were found among mice not maintained under standardized conditions such as alternating 12-hour cycles of light (6 AM to 6 PM) and dark and limited exposure to external stimuli.

Our experiments were designed so that the optimal conventional therapies used would achieve maximal leukemic cell-kill, sufficient to reduce the number of viable leukemia cells surviving therapy to approximately one cell per mouse. Had additional therapeutic benefit been derived via the application of chronopharmacologic concepts related to dosing and scheduling in standardized mice, we should have observed significantly increased cure rates. Under the carefully controlled conditions of the therapy trials reported, we failed to show any therapeutic advantage in mice bearing L1210 leukemia treated with either ara-C or CPA using chronochemotherapy previously reported by others to be useful.

REFERENCES

1. HALBERG F. Physiologic 24-hour periodicity: general and procedural considerations with reference to the adrenal cycle. Ztsch Vit Horm Fermentforsch 10:225–296, 1959.
2. HAUS E, HALBERG F, KUHL J, ET AL. Chronopharmacology in animals. Chronobiologia 1:122–156, 1974.
3. SCHEVING LE, MAYERSBACH H, and PAULY JE. An overview of chronopharmacology. J Eur Toxicol 7:203–227, 1974.
4. SCHEVING LE, and PAULY JE. Circadian phase relationships of thymidine-^{3}H uptake, labeled nuclei, grain counts, and cell division rate in rat corneal epithelium. J Cell Biol 32:677–683, 1967.
5. PAULY JE, SCHEVING LE, BURNS ER, ET AL. Circadian rhythm in DNA synthesis in mouse thymus: effect of altered lighting regimens, restricted feeding, and presence of Ehrlich ascites tumor. Anat Rec 184:275–284, 1976.
6. BURNS ER, SCHEVING LE, PAULY JE, ET AL. Effect of altered lighting regimens, time-limited feeding, and presence of Ehrlich ascites carcinoma on the circadian rhythm in DNA synthesis of mouse spleen. Cancer Res 36:1538–1544, 1976.
7. SCHEVING LE, BURNS ER, and PAULY JE. Can chronobiology be ignored when considering the cancer problem? *In* Proceedings of the 3rd International Symposium on the Detection and Prevention of Cancer. New York, Marcel Dekker, 1977, pp 1063–1079.
8. ROSENE G, LEE J-K, KUHL JFW, ET AL. Circadian chronotherapeutic index: product of adriamycin-associated mouse breast-cancer-shrinkage and survival time augmentation. Int J Chronobiol 1:354–355, 1973.
9. HAUS E, FERNANDES G, KUHL JFW, ET AL. Murine circadian susceptibility rhythm to cyclophosphamide. Chronobiologia 1:270–277, 1974.
10. HAUS E, HALBERG F, SCHEVING LE, ET AL. Increased tolerance of leukemic mice to arabinosyl cytosine with schedule adjusted to circadian system. Science 177:80–82, 1972.
11. SCHEVING LE, HAUS E, KUHL JFW, ET AL. Close reproduction by different laboratories of circadian rhythm in 1-β-D-arabinofuranosylcytosine tolerance by mice. Cancer Res 36:1133–1137, 1976.
12. SCHEVING LE, BURNS ER, PAULY JE, ET AL. Survival and cure of leukemic mice after circadian optimization of treatment with cyclophosphamide and 1-β-D-arabinofuranosylcytosine. Cancer Res 37:3648–3655, 1977.
13. SKIPPER HE, SCHABEL FM, JR, and WILCOX WS. Experimental evaluation of potential anticancer agents. XXI. Scheduling of arabinosylcytosine to take advantage of its S-phase specificity against leukemia cells. Cancer Chemother Rep 51:125–165, 1967.
14. SCHABEL FM, JR, GRISWOLD DP, JR, LASTER WR, JR, ET AL. Quantitative evaluation of anticancer agent activity in experimental animals. Pharmacol Ther 1:411–436, 1977.
15. FINNEY DJ. The comparison of effectiveness. *In* Probit Analysis, 3rd ed. London, Cambridge University Press, 1971, pp 100–124.
16. SCHABEL FM, JR. Synergism and antagonism among antitumor agents. *In* Pharmacological Basis of Cancer Chemotherapy. Baltimore, Williams and Wilkins Co, 1975, pp 595–621.
17. BROCKMAN RW. Circumvention of resistance. *In* Parmacological Basis of Cancer Chemotherapy. Baltimore, Williams and Wilkins Co, 1975, pp 691–711.
18. BROCKMAN RW. Mechanisms of resistance. *In* Antineoplastic and Immunosuppressive Agents, Part I (Sartorelli AC, and Johns DG, eds). Berlin, Springer-Verlag, 1974, pp 352–410.
19. SKIPPER HE, SCHABEL FM, JR, MELLETT LB, ET AL. Implications of biochemical, cytokinetic, pharmacologic, and toxicologic relationships in the design of optimal therapeutic schedules. Cancer Chemother Rep 54:431–450, 1970.
20. WILKOFF LJ, DULMADGE EA, and LLOYD HH. Kinetics of effect of 1-β-D-arabinofuranosylcytosine, its 5'-palmitoyl ester, 1-β-D-arabinofuranosyluracil, and tritiated thymidine on the viability of cultured leukemia L1210 cells. J Natl Cancer Inst 48:685–695, 1972.

Combination Chemotherapy With *cis*-Dichlorodiammineplatinum(II) and Adriamycin for Testicular Cancer Refractory to Vinblastine Plus Bleomycin [1]

Brief Reports and Preliminary Communications

Lawrence H. Einhorn* and Stephen D. Williams [2]

In recent years, combination chemotherapy regimens have produced major gains in remission rate and survival in disseminated testicular cancer (1–3). One of the most widely used regimens has been vinblastine plus bleomycin (1). Since 1974 at Indiana University, primary chemotherapy for disseminated testicular cancer has consisted of *cis*-dichlorodiammineplatinum(II) (DDP), vinblastine, and bleomycin.

During this same time period, we have had the opportunity to evaluate patients who failed to respond to vinblastine plus bleomycin. Since these patients were refractory to adequate doses of vinblastine plus bleomycin, they were clearly not candidates for DDP, vinblastine, and bleomycin. DDP has significant activity as a single agent in testicular cancer (4). Adriamycin also has been shown to have activity in testicular cancer (5) and may be synergistic when combined with DDP (6). This report describes our results with adriamycin and DDP in refractory germinal neoplasms.

MATERIALS AND METHODS

Ten patients with disseminated testicular cancer, who were all refractory to prior vinblastine plus bleomycin, were treated. Table 1 summarizes the pertinent clinical data. None of these patients had achieved complete remission (CR) with prior therapy. All patients had massive pulmonary disease.

DDP was given in a dosage of 20 mg/m^2 for 5 consecutive days every 3 weeks. Two patients received three courses, six had four courses, one had five courses, and one had seven courses of the drug.

Adriamycin was given in a dosage of 60 mg/m^2 iv on the first day of DDP therapy every 3 weeks. The adriamycin dose was reduced by 20% in those patients who had had prior radiotherapy. Maintenance therapy with adriamycin alone was given after completion of DDP until a total dose of 550 mg/m^2 of adriamycin was reached. Only one patient (No. 10) has received maintenance therapy with DDP (75 mg/m^2 once a month) plus adriamycin.

CR was defined as the disappearance of all disease for a minimum of 1 month. Partial remission (PR) was defined as a $> 50\%$ reduction in measured lesions.

RESULTS

All of the ten patients responded objectively to combination chemotherapy with DDP plus adriamycin. However, only one patient achieved a CR. This patient relapsed after completion of maintenance adriamycin, and then had a second CR with DDP alone. The median duration of remission was 5 months and the median survival will be between 8 and 9½ months.

DDP therapy was stopped because of stabilization of response with no further disease regression. Only one patient (No. 4) progressed while receiving DDP. This patient received seven courses of DDP plus adriamycin with further regression with each course. However, when he was admitted for an eighth course, he exhibited progressive pulmonary metastases.

Most relapses occurred in the areas of original disease; however, three patients relapsed in the central nervous system as a new anatomic disease site. No patient achieved further remission after relapse except patient 8 who had a second CR with DDP.

All patients experienced alopecia, nausea, and vomiting. Nephrotoxic effects were mild, with only one patient having a serum creatinine level > 1.5 mg/100 ml. No patient had congestive heart failure or clinically significant hearing loss. Myelosuppression was primarily limited to granulocytopenia, with the median nadir of the granulocyte count being 1.1

[1]Received Feb 3, 1978; revised June 19, 1978; accepted July 20, 1978.

[2]Department of Hematology-Oncology, Indiana University Medical Center, Indianapolis.

**Reprint requests to:* Lawrence H. Einhorn, MD, Department of Hematology-Oncology, Indiana University Medical Center, Emerson Hall, Rm 435, 1100 W Michigan, Indianapolis, Ind 46202.

TABLE 1.—Clinical patient data

Patient No.	Tumor type	Prior therapy*	Extent of disease	Response	Duration (mos)	Survival (mos)
1	Embryonal	*a.* RND + radiotherapy *b.* VB *c.* Actinomycin D + cyclophosphamide + vincristine	Massive bilateral pulmonary metastases	PR	7	10
2	Choriocarcinoma: primary mediastinal	VB	Bilateral cervical nodes, large mediastinal mass, and hepatic	PR	3	5
3	Teratocarcinoma	VB	Peritracheal mass and retroperitoneal nodes	PR	5	11
4	Embryonal	*a.* Actinomycin D *b.* Mithramycin *c.* Bleomycin-COMF *d.* VB	Cervical nodes and massive bilateral pulmonary metastases	PR	5	9
5	Embryonal: primary mediastinal	*a.* Radiotherapy *b.* VB	Large mediastinal mass	PR	3	4
6	Teratocarcinoma	*a.* Actinomycin D + cyclophosphamide *b.* Radiotherapy *c.* VB	Massive bilateral pulmonary metastases	PR	5	7
7	Teratocarcinoma	*a.* RND + radiotherapy *b.* VB *c.* Actinomycin D *d.* Mithramycin	Massive bilateral pulmonary metastases	PR	4	6
8	Teratocarcinoma	*a.* RND + radiotherapy *b.* Actinomycin D *c.* VB	Massive pulmonary metastases	CR	13	26
9	Teratocarcinoma	VB	Retroperitoneal nodes and massive bilateral pulmonary metastases	PR	7	9+
10	Teratocarcinoma	*a.* RND + radiotherapy *b.* VB	Cervical nodes and massive bilateral pulmonary metastases	PR	5+	6+

*RND = retroperitoneal node dissection; VB = vinblastine + bleomycin; COMF = cyclophosphamide, vincristine, methotrexate, and 5-fluorouracil.

$\times 10^3/mm^3$. There were no granulocytopenic infections. No patient developed a platelet count $< 100{,}000/mm^3$. Three patients required transfusion; these three patients all had received prior radiotherapy. Generally, the patients with prior radiotherapy had similar granulocyte count nadirs but a longer duration of granulocytopenia than those patients without prior radiotherapy.

DISCUSSION

This combination regimen produced one CR and nine PRs in ten patients with advanced refractory testicular cancer. Although it was disappointing that we observed only one CR, this patient population was very refractory. In addition to progression with vinblastine plus bleomycin, six patients had had prior radiotherapy and five had failed to respond to at least one other chemotherapeutic regimen in addition to vinblastine plus bleomycin. All ten patients had advanced pulmonary disease (mediastinal mass or pulmonary nodules > 2 cm) and three also had advanced abdominal disease (palpable abdominal mass or hepatic metastases). Six patients had teratocarcinoma and one had choriocarcinoma. One of the three embryonal carcinomas was a mediastinal germinoma. Six patients had elevated alpha-fetoprotein levels. All of these factors have been shown to be unfavorable prognostic indicators in previous studies (2,3,7).

Two other studies have been reported utilizing adriamycin and DDP in a variety of neoplastic diseases. Higby et al used 75 mg/m^2 of adriamycin plus 20 mg/m^2 of DDP for 5 consecutive days every 28 days and achieved two CRs and one PR in three patients with testicular cancer (8). These three

patients had received prior chemotherapy with actinomycin D and vinblastine plus bleomycin. Vogl et al treated five testicular cancer patients with 50 mg/m^2 of adriamycin and 50 mg/m^2 of DDP every 2–3 weeks, and achieved only one PR in five patients (6). These numbers are small, but these two studies, as well as this study, suggest that there may be a therapeutic advantage to 5-day courses of DDP in testicular cancer.

This two-drug regimen produced clinically significant remissions with acceptable toxicity in a very poor-risk patient population. These results were felt to be amply encouraging to warrant investigation of adriamycin as first-line chemotherapy for disseminated testicular cancer in combination with our regimen of DDP, vinblastine, and bleomycin (3). This four-drug regimen is currently being evaluated in a three-arm randomized prospective study with our previous DDP-vinblastine-bleomycin regimen (3), the third arm being the same three-drug regimen utilizing a lower dose (0.3 mg/kg) of vinblastine.

REFERENCES

1. SAMUELS ML, JOHNSON DE, and HOLOYE PY. Continuous intravenous bleomycin (NSC-125066) therapy with vinblastine (NSC-49842) in stage III testicular neoplasia. Cancer Chemother Rep 59:563–570, 1975.
2. CVITKOVIC E, CHENG E, WHITMORE WF, ET AL. Germ cell tumor chemotherapy update. Proc Am Assoc Cancer Res and ASCO 18:324, 1977.
3. EINHORN LH, and DONOHUE J. *Cis*-diamminedichloroplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. Ann Intern Med 87:293–298, 1977.
4. HIGBY DJ, WALLACE HJ, JR, ALBERT DJ, ET AL. Diaminodichloroplatinum: a phase I study showing responses in testicular and other tumors. Cancer 33:1219–1225, 1974.
5. MONFARDINI S, BAJETTA E, MUSUMECI R, ET AL. Clinical use of adriamycin in advanced testicular cancer. J Urol 108:293–296, 1972.
6. VOGL S, OHNUMA T, PERLOFF M, ET AL. Combination chemotherapy with adriamycin and *cis*-diamminedichloroplatinum in patients with neoplastic diseases. Cancer 38:21–26, 1976.
7. SAMUELS ML, LANZOTTI VJ, HOLOYE PY, ET AL. Stage III testicular cancer: complete response by substage to velban plus continuous bleomycin infusion (VB-3). Proc Am Assoc Cancer Res and ASCO 18:146, 1977.
8. HIGBY DJ, WILBUR D, WALLACE HJ, JR, ET AL. Adriamycin-cyclophosphamide and adriamycin-*cis*-dichlorodiammineplatinum(II) combination chemotherapy in patients with advanced cancer. Cancer Treat Rep 61:869–873, 1977.

Interstitial Pulmonary Fibrosis as a Complication of Prolonged Methyl-CCNU Therapy [1,2]

Brief Reports and Preliminary Communications

William Lee,* Robert P. Moore, and Galen L. Wampler [3]

Methyl-CCNU is a new potent chemotherapeutic agent useful against a variety of human tumors. To our knowledge, there have been no previous reports of pulmonary toxic reactions to this drug. We present here a report of a patient in whom prolonged methyl-CCNU therapy resulted in a remarkable control of widespread malignant melanoma until the course was complicated by diffuse interstitial fibrosis thought to be secondary to methyl-CCNU. Cessation of methyl-CCNU therapy and treatment with corticosteroids seemed to improve the patient's pulmonary status but re-appearance of the melanoma led to death.

CASE REPORT

The patient (age, 45 years) was transferred to McGuire Veterans' Administration Hospital in June 1977 for evaluation of an abnormal chest roentgenogram. His illness began in August 1960 when he had a "mole" removed from his right lower leg, pathologically interpreted as "hemorrhagic nevus." In August 1961, a needle aspirate of a right inguinal node was positive for malignant melanoma. The patient underwent a right superficial and deep groin dissection on August 10, 1961. During the operation, he received 108 mg (1.5 mg/kg) of melphalan infused into his right external iliac artery. In September 1969, he had a wide local excision for a recurrence on his right lower leg. By February 1971, satellite lesions appeared around the skin graft in the same area, and following an appropriate workup, the patient had a right hemipelvectomy. The next recurrence was documented in November 1974 by pleural biopsy after the demonstration of densities in both lung fields along the pleural surface (fig 1). A bone marrow biopsy was also positive for metastatic melanoma.

On November 22, 1974, he began receiving methyl-CCNU, 300 mg orally every 6 weeks, which produced a good partial remission with disappearance of most of the pleural-based metastases after receiving treatment for 5 months. Treatment was continued with methyl-CCNU (100–300 mg, depending upon his hematologic status) every 6 weeks until December 13, 1976, when he complained of a nonproductive cough and some dyspnea on exertion. A 2-week course of ampicillin for presumed bronchitis brought some improvement. A chest roentgenogram at that time showed continued shrinkage of the pleural masses first observed in 1974 and bilateral nonspecific interstitial infiltrates. At this time, the patient had received a total of 4.1 g of methyl-CCNU. A repeat bone marrow examination (January 1977) was negative for tumor. During the next 5 months he received an additional 0.4 g of methyl-CCNU and his symptoms gradually progressed. A repeat chest roentgenogram (June 1977) showed an increase in the number of infiltrates, some of which had become confluent (fig 2). The patient was then admitted for further evaluation of the pulmonary disease.

Upon admission, he complained of dyspnea on exertion during average walking (with prosthetic leg) and a cough minimally productive of grey-white sputum. He denied fever, chills, or exposure to tuberculosis or organic or chemical irritants but admitted to smoking about one-half pack of cigarettes per day. A physical examination revealed a thin, slightly cachectic white man in no acute distress. His blood pressure was 100/70, the pulse rate was 88 and regular, the temperature was 37°C orally, and the respiratory rate ranged from 20 to 24/minute. There was some dullness in the left base associated with decreased breath sounds in the left posterior and anterior lung fields. A pleural friction rub was heard along the left posterior axillary line at the base. Cellophane rales were heard throughout both lung fields. The liver was 11 cm in total span without masses. A well-healed right hemipelvectomy scar was noted and the neurologic examination appeared normal. The laboratory data included Hgb, 15.1 g/100 ml, and wbc count, 10,400/mm^3 with 65% polymorphonuclear neutrophils, 4% band cells, 22% lymphocytes, 3% eosinophils, and 6%

[1]Received Apr 20, 1978; revised June 29, 1978; accepted July 6, 1978.

[2]Supported in part by Public Health Service grant CA-10572 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Department of Medicine, McGuire Veterans' Administration Hospital, Richmond, Va.

*Reprint requests to: William Lee, MD, Box 243, Randolph-Minor Hall, Medical College of Virginia, Richmond, Va 23298.

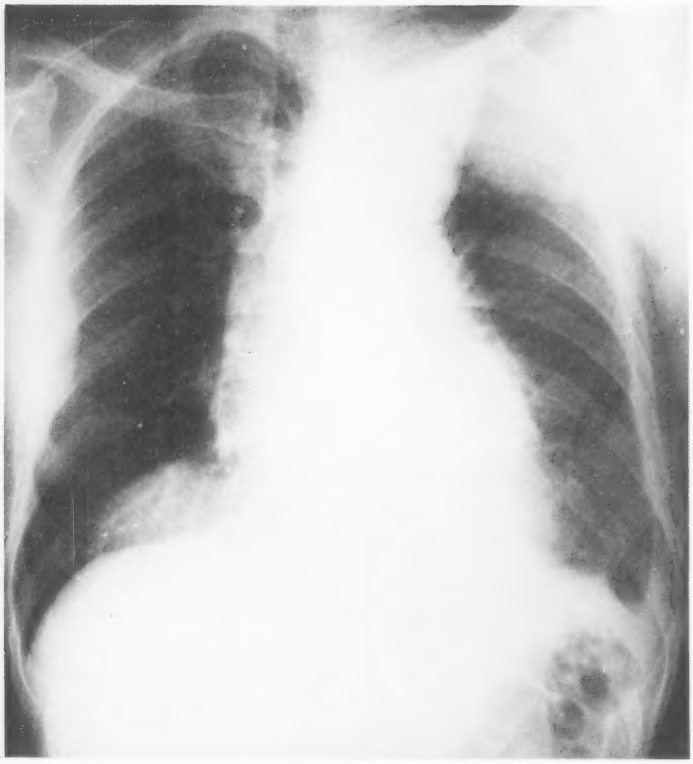

FIGURE 1.—Posteroanterior chest roentgenogram in Oct 1974 prior to therapy with methyl-CCNU. There are multiple pleural-based densities. A pleural biopsy showed metastatic melanoma.

FIGURE 2.—Chest roentgenogram showing diffuse interstitial fibrosis. Also note resolution of pleural-based masses 32 mos after roentgenogram shown in fig 1.

monocytes. The blood chemistries were normal except for elevation of the serum alkaline phosphatase to 145 units (normal = up to 105). The resting pO_2 was 56 mm Hg, the pCO_2 was 32 mm Hg, and the pH was 7.46. Pulmonary function studies showed a marked reduction in vital capacity, impaired diffusion capacity, and postexercise hypoxemia. Multiple cytologic studies of the sputum were negative for malignant cells and cultures later proved negative for fungi and mycobacteria. Giemsa-stained smears of the sputum showed a predominance of neutrophils which were loosely distributed; however, there were focal areas showing lymphocytes, eosinophils, alveolar histiocytes, and a few mast cells. The probability of an adverse drug reaction was then considered.

On June 12, 1977, the patient underwent an open lung biopsy which showed diffuse fibrosis of the alveolar septae, "desquamated" cells in a few alveoli, and cuboidalization of the alveolar lining cells (fig 3). Other areas showed dense fibrous tissue, obliteration of alveolar spaces, and thick-walled pulmonary arteries. No normal-appearing alveoli could be found and there was no evidence of metastatic melanoma. The Masson-trichrome stain indicated that the fibrous tissue was quite young without much collagen formation. These findings are compatible with diffuse interstitial pulmonary fibrosis. The patient began receiving prednisone, 60 mg/day × 30 days, and then 30 mg/day. By August, the patient had no dyspnea and had an increased appetite with a weight gain of 15 pounds. A chest roentgenogram showed partial clearing of the interstitial markings.

He was re-admitted on September 30, 1977, complaining of a right retro-orbital headache for 6 days. Questionable papilledema was noted in the left fundus. Before diagnostic tests could be performed, he had a generalized seizure and died.

An autopsy revealed marked generalized cerebral edema with a 4-cm right frontal lobe necrotic tumor extending into the right lateral ventricle, microscop-

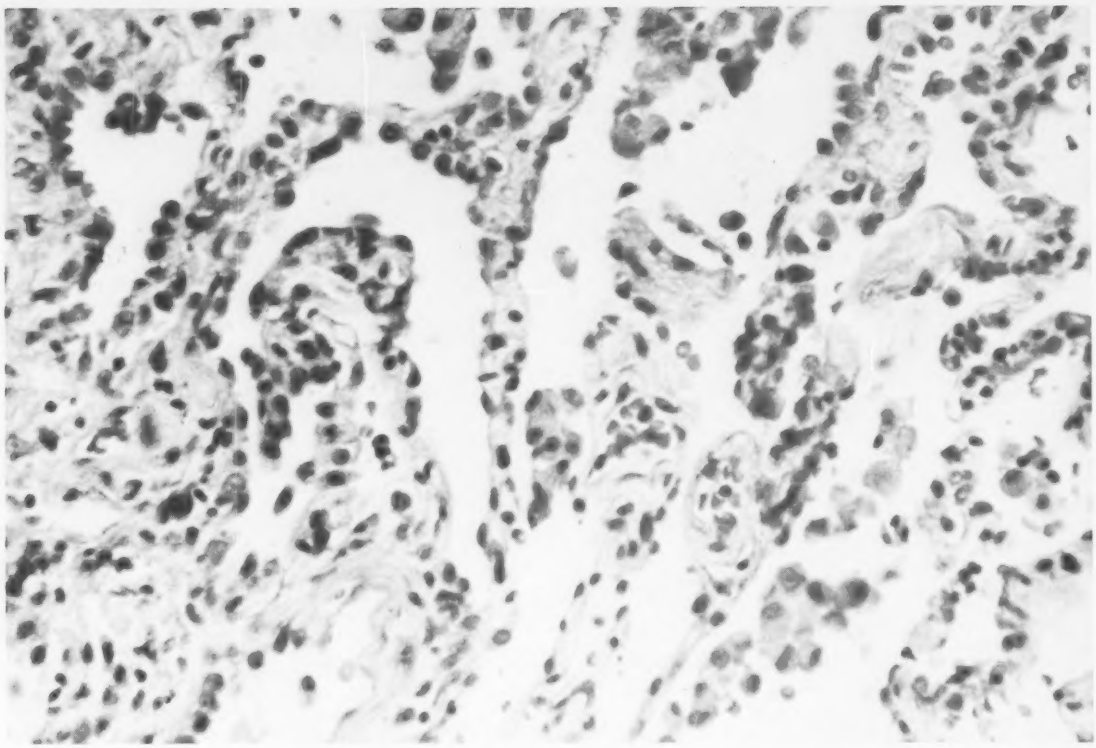

FIGURE 3.—Open-lung biopsy showing increased fibrous tissue in the alveolar septae, cuboidalization of alveolar lining cells, and small collections of "desquamated" cells in some alveoli.

ically confirmed as metastatic melanoma. Microscopic sections of the pulmonary parenchyma again showed dense areas of fibrous tissue without evidence of malignancy, but small areas of normal-appearing alveoli could be found and there was little evidence of alveolitis. Both pleural surfaces were markedly thickened with fibrosis and there was considerable involvement of the left parietal pleura with melanoma. There was no evidence of tumor elsewhere in the body.

DISCUSSION

Because of the temporal relationship between methyl-CCNU therapy and the development of pulmonary fibrosis in this patient, this drug appeared to be implicated etiologically. While diffuse interstitial fibrosis secondary to methyl-CCNU has not been previously reported, BCNU has been reported to have pulmonary toxic effects (1,2). In this case report, except for one extra-arterial injection of melphalan 16 years previously and a brief course of ampicillin after his symptoms had started, the patient had had no prior exposure to radiation or drugs other than methyl-CCNU that could cause pulmonary toxic effects. Most drugs known to cause pulmonary fibrosis do so after chronic use and are dose-related. This patient's symptoms and initial roentgenographic abnormality were first noted after a cumulative dose of 4.1 g over a 25-month period. Patients treated with methyl-CCNU often do not continue to receive therapy for several years because of the limiting nature of their primary disease. With methyl-CCNU being used more frequently in adjuvant protocols (for example, colon and rectal carcinomas), the problem may be seen more frequently.

REFERENCES

1. WASSERMAN TH. The nitrosoureas: an outline of clinical schedules and toxic effects. Cancer Treat Rep 60:709–711, 1976.
2. CRITTENDEN D, TRANUM BL, and HAUT A. Pulmonary fibrosis after prolonged therapy with 1,3-bis(2-chloroethyl)-1-nitrosourea. Chest 72:372–373, 1977.

Plasma Levels and Urinary Excretion of Filterable Platinum Species Following Bolus Injection and Iv Infusion of *cis*-Dichlorodiammineplatinum(II) in Man [1,2]

Brief Reports and Preliminary Communications

Thomas F. Patton,* Kenneth J. Himmelstein, Robert Belt, Steve J. Bannister, Larry A. Sternson, and A. J. Repta [3,4]

cis-Dichlorodiammineplatinum(II) (CDDP) is an active divalent coordination complex of platinum currently undergoing clinical trials. Several studies have investigated the distribution of divalent platinum coordination complexes in the body following their administration to both animals (1–3) and man (4,5). Most of the reported methods have monitored the disposition of total platinum (1–8) and were not able to discriminate between protein-bound and non-protein-bound species (4). The ability to discriminate between these species becomes particularly important when one considers that the protein-bound materials are apparently inactive and that within 3 hours after administration of CDDP only about 10% of the dose remains unbound in the blood (4,9,10). Using in vitro colony-forming units in culture (CFU-C) of human marrow precursor cells, we have found that the active cytotoxic species of CDDP is in the unbound fraction. The protein-bound species appears to have little if any activity (Belt R, and Holdener E, unpublished results). Therefore, if it is assumed that the drug exerts chemotherapeutic activity only in the unbound form, as is the case with other drugs (11), meaningful pharmacokinetic studies must be able to evaluate the disposition behavior of the drug in this state.

Recent analytic developments (9,12) utilizing centrifugal ultrafiltration and atomic absorption spectrophotometry have provided the methodology for the separation and subsequent analysis of clinically relevant concentrations of filterable platinum species following the administration of CDDP.[5] In this report, this analytic methodology has been used to determine filterable platinum species in both plasma and urine following iv administration of CDDP to man. While previous reports have dealt only with the pharmacokinetics of the iv bolus administration of CDDP, we present data on the distribution of filterable platinum species following an iv bolus as well as a 6-hour iv infusion of CDDP. Pharmacokinetic analysis of our data reveals a rapid decline in plasma concentrations consistent with previous reports (4) and substantial differences in plasma level decline and urinary excretion patterns when the bolus and infusion routes of administration are compared.

MATERIALS AND METHODS

Patients and Protocol

Four patients received seven doses of CDDP. Courses were repeated at 3-week intervals in patients who received more than one dose, provided that all evidence of drug-related toxicity was absent. Three administrations (two patients) were via 15-minute iv bolus injections and four administrations (two patients) were via 6-hour iv infusion. The dose of CDDP in all patients was 100 mg/m^2. CDDP was supplied by the National Cancer Institute and was administered in sterile, normal saline solution.

All patients selected for study had histologically proven malignancies and a patent lower urinary tract as demonstrated by iv pyelogram and by rectal examination to include benign prostatic hypertrophy. All patients had normal renal function as defined by a BUN of ≤ 20 mg/100 ml, serum

[1]Received Feb 22, 1978; revised May 25, 1978; accepted July 6, 1978.

[2]Supported in part by Public Health Service grants CA-09242 (National Cancer Institute) and RR-00828 and RR-07037 (Division of Research Resources) from the National Institutes of Health, Department of Health, Education, and Welfare.

[3]Departments of Pharmaceutical Chemistry (*T. F. Patton, S. J. Bannister, L. A. Sternson,* and *A. J. Repta*) and Chemical and Petroleum Engineering (*K. J. Himmelstein*), The University of Kansas, Lawrence; and Department of Medical Oncology (*R. Belt*), The University of Kansas Medical Center, Kansas City.

[4]The technical assistance of Ms. Janice Stevens and Lyn Cain, RN, is gratefully acknowledged.

**Reprint requests to:* Thomas F. Patton, PhD, Pharmaceutical Chemistry Laboratory, McCollum Laboratories, 2065 Ave "A"-Campus West, The University of Kansas, Lawrence, Kan 66044.

[5]Using this method, the species being monitored is either non-protein-bound platinum or platinum species which are bound to macromolecules with a molecular weight $< 50{,}000$. We have demonstrated (ref 9) that ultrafiltration removes $> 98\%$ of proteinaceous material, ie, species responding to Biuret assay.

TABLE 1.—Filterable platinum species in plasma and associated terminal half-lives following bolus injection and 6-hr infusion of 100 mg/m² of CDDP

Patient No.	Mode of administration*	Concentration (ng/ml) Time (mins)† 0	15	30	45	60	120	Half-life (mins)
1a	Bolus	4880	2550	1410	1060	710	260	53.5
1b‡	Bolus	2890	2280	1690	1100	830	280	45.8
2	Bolus	3440	1690	580	780	560	100	32.0
							Mean (SE)	43.8 (6.3)
3a	6-hr infusion	530	230	74	87	45	—	17.5
3b‡	6-hr infusion	540	320	140	53	61	18	26.0
4a	6-hr infusion	—	170	190	120	64	28	37.1
4b‡	6-hr infusion	460	240	170	160	62	12	23.1
							Mean (SE)	25.9 (4.1)

* Described under Materials and Methods section.
† Time after completion of administration of CDDP.
‡ Drug administered on 2 occasions to same patient.

creatinine of ≤ 1.5 mg/100 ml, normal urinalysis, and creatinine clearance of ≥ 70 ml/minute (range, 70–90 ml/minute). All blood values, including protein levels, were within normal limits, indicating no significant organ abnormalities in these patients. Patients must have recovered from toxic effects of prior chemotherapy (minimum of 3 weeks) and had to have a wbc count $> 4000/mm^3$ and platelet count $> 130,000/mm^3$. All patients had an expected survival of at least 6 weeks. Patients were hospitalized, prehydrated with 2000 ml of 5% dextrose in ½ normal saline over 12–24 hours, and maintained on iv fluids for a minimum of 24 hours after receiving CDDP.

Blood samples were collected immediately following completion of administration, at 15, 30, 45, 60, and 120 minutes after injection, and at the same time points after the infusion was stopped. Total urine was collected for 24 hours after initiation of drug administration. Although later samples were obtained, the levels of filterable platinum were below the detection limits of the assay after 2 hours in the case of plasma and after 24 hours in the case of urine.

Analytic Method

The analytic procedure used to measure filterable platinum species has been described in detail elsewhere (12). Briefly, protein-bound and filterable platinum were separated by centrifugal ultrafiltration using Centriflo CF-50 filters (Amicon Corp). Platinum in the ultrafiltrate was converted by reaction with ethylenediammine to a cationic-complex which was collected on cation-exchange resin-impregnated paper discs (Whatman, Inc). In this form, the samples could be stored indefinitely without loss. To complete the analysis, the platinum was eluted from the disc with 5 N hydrochloric acid (250 μl) and an aliquot (1–2 μl) of this solution was then analyzed by flameless atomic absorption spectrophotometry. Each sample was run in triplicate; reproducibility was found to be $\pm$ 2%.

Data Analysis

Plasma-decay curves for bolus injections and 6-hour infusions (after termination of infusion) were subjected to pharmacokinetic analysis using a nonlinear regression program on a Honeywell 66/60 digital computer. In the case of bolus administration, correlation coefficients indicated that the biexponential two-compartment model clearly provided the best fit to the data (correlation coefficients: monoexponential < 0.8 and biexponential > 0.9). With the infusion data, terminal slopes of plasma decline were virtually identical regardless of whether a monoexponential or biexponential fit was employed. Following infusion, the ability to discern the two-compartment characteristics of a drug is usually decreased (13).

RESULTS

Plasma data for both iv bolus and infusion methods of administration along with their associated computer-generated β-phase half-lives are shown in table 1. Early time data were not sufficient to

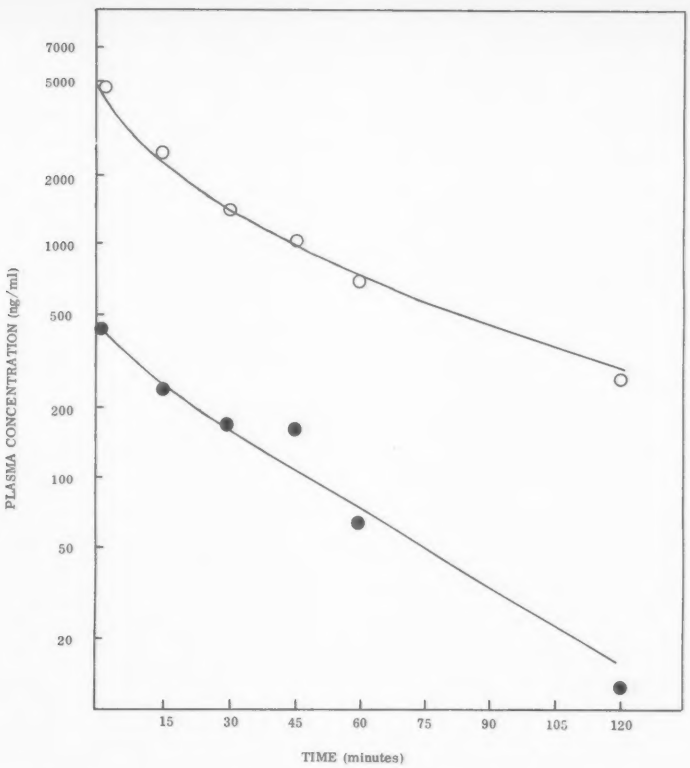

FIGURE 1.—Typical plasma level decline of filterable platinum species following bolus injection (○, patient 1a) and 6-hr infusion (●, patient 4b) of 100 mg/m² of CDDP.

perform a detailed pharmacokinetic analysis of the initial plasma decline. Since sufficient samples were not obtained during infusion to discern whether or not the steady-state had been attained, it is possible that distribution continued to occur after termination of infusion (fig 1 shows a typical example of the plasma levels of filterable platinum). Table 2 shows the comparative percent of dose recovered in the urine in 24 hours.

DISCUSSION

Plasma levels of filterable platinum species after bolus injection decline in an apparent biphasic mode with a terminal half-life of 32–53.5 minutes. When measuring the decline in *total* platinum species in plasma after iv injection, DeConti et al (4) observed a biphasic decline with an *initial* half-life of 25–49 minutes. Thus, it appears that the initial plasma decline observed by DeConti et al was a result of the rapid plasma clearance of filterable material. This was alluded to but could not actually be directly confirmed with the analytic methodology used in that study. This observation is significant in understanding the disposition of CDDP in the body, especially if it is confirmed that only the unbound material is active. Furthermore, our observation that filterable platinum species undergo a biphasic decline in plasma indicates that a distribution phenomenon occurs with filterable as well as total platinum species.

The nonfilterable material which remains in the body for substantially longer periods of time than the filterable material could serve as a depot by slowly releasing the active species to the tissues. Until this active species is identified however, and therapeutic and toxic levels are ascertained, it appears unprofitable to speculate on the significance of the long half-life of total platinum as observed by DeConti et al (4). However, since the filterable species declines at such a rapid rate and accounts for elimination of such a large fraction of

TABLE 2.—Cumulative % of dose as filterable platinum species recovered in urine in 24 hrs following iv bolus injection and 6-hr iv infusion of 100 mg/m² of CDDP

Patient No.	Cumulative % of dose as free platinum species
Iv injection	
1a	51.3
1b	48.1
2	46.0
6-hr iv infusion	
3a	81.9
3b	66.6
4a	75.5
4b	75.6

the administered dose, it is imperative that this species be accounted for in developing CDDP dosing regimens.

In addition to the observation of a rapid plasma decline in filterable platinum species noted in this study, several rather unusual observations regarding the pharmacokinetic behavior of this species can be made by comparing the two different modes of administration employed in this study. First, the half-life of the filterable material in the plasma after termination of infusion is substantially shorter than that observed after the bolus injection ($P \leq 0.05$). Second, the fraction of the total dose of CDDP excreted as filterable platinum in the urine in 24 hours is substantially greater after a 6-hour infusion than after a bolus injection; the mean percent excreted is 74.9% ± 3.1% for infusions and 49.5% ± 5% for bolus injections (these two means are significantly different at $P \leq 0.05$). With the limited amount of data presently available, it is not possible to state with certainty the reason for these differences, although explanations can be postulated. The differences may be due to the binding behavior and reactivity of various platinum species derived from CDDP in blood and tissues. Alternatively, the observed differences in half-lives between bolus injection and infusion may arise if a steady-state is not reached during the period of infusion. Based on the half-life of the filterable material, one would intuitively assume that plateau levels would easily be reached during a 6-hour infusion. If however, plateau levels were not reached, continued distribution following termination of the infusion could account for the shorter half-life observed following infusion administration.

It is important to note however that regardless of the reason for the observed differences, the rate at which filterable platinum levels decline as a function of route of administration could have important implications in establishing optimal CDDP dose regimens and in evaluating drug-related toxic effects. Clinicians presently using CDDP in clinical trials should be aware of such differences until more mechanistic studies evaluating the disposition patterns of various platinum species under a variety of dosing conditions are available.

REFERENCES

1. LANGE RC, SPENCER RP, and HARDEN HC. Synthesis and distribution of a radiolabeled antitumor agent: *cis*-dichlorodiammineplatinum(II). J Nucl Med 13:328–320, 1972.
2. WOLF W, MANAKA RC, and INGALLS RD. Radiopharmaceuticals in clinical pharmacology. Radiopharm Labeled Compd 2:205–221, 1973.
3. TAYLOR DM, JONES JD, and ROBINS AB. Metabolism of platinum [^{14}C] ethylenediamine dichloride in the rat. Biochem Pharmacol 22:833–839, 1973.
4. DECONTI RC, TOFTNESS BR, LANGE RC, ET AL. Clinical and pharmacological studies with *cis*-diamminedichloroplatinum(II). Cancer Res 33:1310–1315, 1973.
5. LITTERST CL, GRAM TE, DEDRICK RL, ET AL. Distribution and disposition of platinum following intravenous administration of *cis*-diamminedichloroplatinum(II) (NSC 119875) to dogs. Cancer Res 36:2340–2344, 1975.
6. MILLER RG, and DOERGER JV. Determination of platinum and palladium in biological samples. Atom Absorp Newsletter 14:66–67, 1975.
7. JONES AJ. Determination of platinum and palladium in blood and urine by flameless atomic absorption spectrophotometry. Anal Chem 48:1472–1474, 1976.
8. LEROY AF, WEHLING ML, SPONSELLER HL, ET AL. Analysis of platinum in biological materials by flameless atomic absorption spectrophotometry. Biochem Med 18:184–191, 1977.
9. BANNISTER SJ, STERNSON LA, REPTA AJ, ET AL. Measurement of free-circulating *cis*-dichlorodiammineplatinum(II) in plasma. Clin Chem 23:2258–2262, 1977.
10. LEH FKV, and WOLF W. Platinum complexes: a new class of antineoplastic agents. J Pharm Sci 65:315–328, 1976.
11. MARTIN BK. Potential effects of the plasma proteins on drug distribution. Nature 207:274–276, 1965.
12. BANNISTER SJ, CHANG Y, STERNSON LA, ET AL. Plasma analysis of free-circulating platinum species derived from *cis*-dichlorodiammineplatinum(II) by atomic absorption spectrophotometry. Clin Chem 24:877–880, 1978.
13. GIBALDI M, and PERRIER D. Pharmacokinetics. New York, Marcel Dekker, Inc, 1975.

Phase I Study of Neocarzinostatin in Patients With Acute Leukemia [1]

Brief Reports and Preliminary Communications

David A. Van Echo* and Peter H. Wiernik [2]

In Japan, neocarzinostatin (NCS) has been used to treat a variety of human cancers including pancreatic (1) and gastric (2). Since 1972, it has been used extensively in acute nonlymphocytic leukemia (ANLL) in which it is purported to have its major clinical utility. NCS has been used alone (complete response [CR] rate, 50%) (3) and in combination with 6-mercaptopurine, vincristine, and prednisone (CR and partial response [PR] rate, 70%) (4), with daunorubicin (CR and PR rate, 78%), and with cyclocytidine (response rate, 29%). Recent data from Japan, however, suggest less antileukemic activity than previously reported (5). Both previously treated and untreated patients were included in these studies. We initiated the present phase I study in an effort to define the proper NCS dose and schedule with which to conduct a phase II trial of NCS in acute leukemia.

MATERIALS AND METHODS

In this study, NCS was diluted in 250 ml of 5% dextrose and water in a foil-covered bottle and was administered by iv infusion over 2 hours. In Japan, for patients with acute leukemia, NCS is given at a dose of 2000–3000 units/m²/day × 5. In this study, it was elected to start the dose at 1000 units/m²/day × 5 and escalate.

Sixteen adult patients with refractory ANLL and one 8-year-old child with acute lymphocytic leukemia (ALL) received 26 courses of NCS. Patients were entered in cohorts of two or more at the following dose levels (all in units/m²/day): 1000 × 5, 1250 × 5, 1500 × 5, 1750 × 5, 2000 × 5, 2500 × 5, 2500 × 10, 3000 × 5, 3000 × 10, 3500 × 5 (one patient), 3500 × 10, and 3500 × 15 (one patient). All patients had received extensive prior chemotherapy. Full supportive care was given to all patients including prophylactic oral nonabsorbable antibiotics (6), empiric or specific parenteral antibiotics (7), prophylactic platelet transfusions when the platelet count fell to < 20,000/mm³, and granulocyte transfusions for infections not controlled by appropriate antibiotics alone. All patients were examined daily and all but one were hospitalized. Complete hemograms were done three times weekly, and liver, renal, and other serum biochemical tests were obtained weekly or more often if indicated clinically. Bone marrow aspirations were performed on all patients prior to entry in the study, on Days 5, 10, and 15 of the first course of NCS therapy, and as clinically indicated thereafter.

The bone marrow response determined subsequent therapy. If toxicity was acceptable and the marrow remained hypercellular with increased numbers of leukemic cells, the dose of NCS was increased in the same patient to the next dosage escalation level. Courses of therapy were continued until patients achieved remission, developed unacceptable toxicity, or had progressive disease. Remission status was determined according to previously published criteria from this institution (8).

RESULTS

Patient characteristics are listed in table 1. Sixteen of the 17 patients were evaluable for toxicity and response. All of the patients had nausea, and vomiting occurred in ten patients (59%) at all dosage levels. Antiemetic drugs had little influence on the suppression of vomiting. A low-grade fever (100°F–101°F) was present on each treatment day in 12 patients (70%), but subsided within 24 hours after the last NCS dose. Early in the study, there were four episodes of fever to 104°F which usually occurred on the first or second treatment day of a course, approximately 2–4 hours after the NCS infusion. The fever was sometimes, but not always, accompanied by severe shaking chills. There were six episodes of severe chills. The onset was 1½–2 hours after the NCS infusion and if left untreated would persist for several hours. It was found that both the fever and chills were easily treated with acetaminophen and diphenhydramine and, in fact,

[1] Received Sept 6, 1977; revised June 14, 1978; accepted June 21, 1978.

[2] Baltimore Cancer Research Center, Division of Cancer Treatment, National Cancer Institute, Md.

Reprint requests to: David A. Van Echo, MD, Baltimore Cancer Research Center, Division of Cancer Treatment, National Cancer Institute, University of Maryland Hospital, 22 S Greene St, Baltimore, Md 21201.

TABLE 1.—Patient characteristics

Characteristic	
No. of patients	16
Men	9
Women	7
Median age in yrs (range)	31.5 (8–71)
No. of patients with—	
ALL	1
ANLL	15
Median No. of previous remissions (range)	1 (0–2)
Median duration of survival from diagnosis to entry in the study in wks (range)	52 (6–144)
Median duration of first remission in wks (range)	24 (0–104)
Median time since last remission in wks (range)	20 (2–50)
No. of patients not previously in remission	4
Median time from end of last treatment to entry in the study in wks (range)	5 (1–22)

could be prevented if the patient was premedicated orally prior to receiving NCS. Once this observation was made, it became routine to premedicate patients with these drugs and no more episodes of fever or chills were observed. One case of hypotension was seen in the child with ALL on the first day of her second course of NCS. This was coincident with a high fever and chill. The previously mentioned medications plus iv fluids returned the blood pressure to normal. NCS therapy was continued in this patient on the succeeding 4 days with premedication, and no further problems were encountered.

Renal function remained unaffected at all dose levels tested. Liver function abnormalities were noted in ten patients prior to entry in the study. The most common causes were previous chemotherapy, presumed leukemic infiltration of the liver, or both. In two patients, the liver function abnormalities progressed. One patient died from fulminating HAA-positive hepatitis with necrosis, and in the other patient the common bile duct was found at autopsy to be partially obstructed by porta hepatic nodes enlarged with leukemic infiltrates. In all of the other patients, the hepatic dysfunction remained stable or improved.

One patient developed a generalized erythematous, non-pruritic rash at the end of her second NCS course which persisted for 2 weeks. No other adverse side effects were noted, specifically, urticaria, stomatitis, arthralgia, diarrhea, or neurologic changes.

No patient achieved a CR. One partial bone marrow response (M-2) was achieved in the patient with ALL for 1 month; however, there was no change in her hepatosplenomegaly and her leukemia proved refractory to a second course of NCS. Marrow hypoplasia was produced at dose levels > 2000 units/m^2/day $\times$ 5 in a sporadic fashion (one of three patients at 2000 units/m^2/day $\times$ 5, one of four patients at 3000 units/m^2/day $\times$ 5, and one of two patients at 3500 units/m^2/day $\times$ 10). An additional three patients had prolonged episodes of marrow aplasia (one of two patients at 3000 units/m^2/day $\times$ 10, one of one patient at 3500 units/m^2/day $\times$ 5, and one of two patients at 3500 units/m^2/day $\times$ 10). Two of these last patients died from complications of their prolonged aplasia and the third had a stormy septic course before the bone marrow regenerated with leukemic cells and alternative chemotherapy was given. Five of the six patients who developed hypoplasia or aplasia did so on an initial exposure to NCS. No patient had any useful antileukemic response to the drug.

Because of poor marrow reserve and the aggressiveness of the leukemic process, it was often necessary to re-treat our patients before recovery was seen in the peripheral blood counts; therefore, meaningful data on hematologic recovery time are not available in this study. Courses of NCS were repeated on an average of every 3 weeks (range, 2–11). Nadir counts were consistent and were comparable for patients treated at doses $>$ and $<$ 2000 units/m^2/day $\times$ 5. The pretreatment wbc count averaged 8800/mm^3 (range, 500–52,000) with a nadir at 7 days (range, 3–12) of 1400/mm^3 (range, 100–6300). The pretreatment platelet count averaged 16,000/mm^3 (range, 5000–46,000) with a nadir on Day 8 (range, 2–12) of 6000/mm^3 (range, 2000–10,000).

DISCUSSION

When given as a 2-hour infusion, as in this study, NCS has few side effects and is well-tolerated by patients. The nausea and vomiting, when present, did not represent a significant problem and the fever with or without chills could be eliminated by premedication with acetaminophen and diphenhydramine. The one episode of hypotension was felt to be secondary to fever and relative hypovolemia. There was no evidence of hepatic or renal dysfunction. This is interesting, because in the preclinical toxicology trials it was found that NCS provoked a fatal antigen-antibody-mediated glomerulonephritis in dogs. Except for the one instance of skin rash, there was no evidence of allergic phenomena from NCS in this study. Therapeutically, there were no meaningful responses.

The above information is, in general, consistent with the experience of other centers which utilized NCS in other dosage schedules. The information concerning hematologic data is more conflicting. In this study, NCS produced marrow hypoplasia at doses > 2000 units/m²/day × 5 and prolonged aplasia at doses ≥ 3000 units/m²/day × 10. When NCS is given as a continuous infusion over 24 hours to patients with acute leukemia, transient hypoplasia is induced at doses ≥ 2000 units/m²/24 hours × 5, but aplasia is not produced until 8000 units/m²/24 hours × 5 is given.[3,4] This could be explained by instability of the drug over time or secondary to its light-sensitivity, increased renal clearance, or decreased uptake of the drug because of the lower peak plasma concentration which is produced by continuous infusion. When given as a rapid iv infusion over 10 minutes to solid tumor patients, little myelosuppression was seen up to doses of 6400 units/m²/day × 5 every 2–3 weeks.[3] This contrasts with the findings of another group which felt that 30-minute infusions of NCS at 2200 units/m²/day × 5 every 3 weeks were dose-limiting in solid tumor patients, especially those with extensive prior chemotherapy (9). For the most part, our study population consisted of end-stage leukemia patients with multiple prior chemotherapy attempts and this, it could be argued, is the reason for the episodes of aplasia.

From this experience, it is concluded that for phase II leukemic studies using a 2-hour infusion of NCS, a starting dose of 2000–3000 units/m²/day × 5 every 3 weeks is reasonable and solid tumor phase II studies using the same schedule should use a more cautious starting dose.

REFERENCES

1. KINAMI Y, MIYAZAKI H, KOYAMA F, ET AL. The treatment of pancreatic carcinoma with neocarzinostatin. J Jpn Soc Cancer Ther 10:502–508, 1975.
2. ANEHA Y, KIKUCHI K, and KANNO H. Administration of an antineoplastic agent of high molecular weight, neocarzinostatin, in cases of gastric cancer. Jpn J Clin Med 32:870–874, 1974.
3. KITAJIMA K, KAMIMURA O, and HIRAKI K. Neocarzinostatin: a new chemotherapeutic approach to acute leukemia. Acta Haematol Jpn 37:316–323, 1974.
4. KITAJIMA K, KAMINURA O, NAGAO T, ET AL. Neocarzinostatin: a new agent active in the treatment of acute leukemia. Bibl Haematol 40:751–752, 1975.
5. MASAOKA T, HASEGAWA Y, YOSHITAKE J, ET AL. Mode of decrease of leukemic cells and leukocytes during neocarzinostatin treatment. Cancer Treat Rep 61:73–75, 1977.
6. SCHIMPFF SC, GREENE WH, YOUNG VM, ET AL. Infection prevention in acute nonlymphocytic leukemia. Laminar air flow room reverse isolation with oral, nonabsorbable antibiotic prophylaxis. Ann Intern Med 82:351–358, 1975.
7. GAYA H, KLASTERSKY J, SCHIMPFF SC, ET AL. Protocol for an international prospective trial of initial regimens in neutropenic patients with malignant disease. Eur J Cancer (suppl) 11:1–4, 1975.
8. WIERNIK PH, and SERPICK AA. Factors affecting remission and survival in adult acute nonlymphocytic leukemia (ANLL). Medicine 49:505–513, 1970.
9. GRIFFIN T, COMIS RL, LOKICH J, ET AL. Neocarzinostatin: phase I study. Proc Am Assoc Cancer Res and ASCO 18:302, 1977.

[3]McKelvey EM. Phase I studies of neocarzinostatin. Annual Report to the Food and Drug Administration. Sept 1977, pp 1–3.

[4]Ohnuma T. Phase I studies of neocarzinostatin. Annual Report to the Food and Drug Administration. Sept 1977, pp 5–16.

Phase II Study of Adriamycin and Bleomycin in Patients With Multiple Myeloma [1]

Brief Reports and Preliminary Communications

John M. Bennett,* Robert Silber, Ediz Ezdinli, Martin Levitt, Martin Oken, Richard F. Bakemeier, John C. Bailar, and Paul P. Carbone [2]

SUMMARY

Twenty-seven patients with far-advanced multiple myeloma, resistant to standard agents, were treated with either adriamycin (eight patients) or bleomycin (19 patients). Adriamycin was given iv at a dose of 60 mg/m² every 3 weeks and bleomycin was administered im at a dose of 15 mg/m² weekly. Drug toxicity was modest. One partial response was seen with each agent, both occurring in "good-risk" patients.

[Cancer Treat Rep 62:1367–1369, 1978]

In the continued search for new effective agents for the treatment of multiple myeloma, the Eastern Cooperative Oncology Group (ECOG) undertook an evaluation of adriamycin and bleomycin in patients refractory to conventional alkylating agents, corticosteroids, and radiation therapy. Adriamycin was chosen because of its proven efficacy in patients with other hematologic malignancies, such as acute leukemia (1) and lymphomas (2), and preliminary results of activity in cyclophosphamide- and/or melphalan-resistant myeloma (3). Bleomycin (also effective in both Hodgkin's disease and non-Hodgkin's lymphoma [4]), with its established marrow-sparing properties, appeared to be worthy of a trial.

MATERIALS AND METHODS

Thirty-two patients with advanced myeloma who had failed to respond to standard agents, including radiation, were entered in this study. Radiation therapy was used only to provide pain relief from bony involvement. Twelve patients received adriamycin alone (11 patients were randomized) and 20 patients received bleomycin (15 patients were randomized). In addition, there was a crossover design for failures after 6 weeks of treatment resulting in one crossover to adriamycin and four crossovers to bleomycin. Patients were stratified retrospectively by both the ECOG performance status (PS) and the "good-risk" and "poor-risk" classifications, using the criteria of the Chronic Leukemia-Myeloma Task Force (5). The "poor-risk" group included any of the following: (*a*) hemoglobin < 7.0 g/100 ml; (*b*) BUN > 40 mg/100 ml; or (*c*) serum calcium > 11.0 mg/100 ml.

Patients were to continue in the study for a minimum of 6 weeks (two courses of adriamycin or six courses of bleomycin). The dose of adriamycin was 60 mg/m² by iv bolus once every 3 weeks and the dose of bleomycin was 15 mg/m²/week im. There was no dosage escalation. Patients were evaluated for response according to the criteria established by the ECOG and the Southeastern Cancer Study Group.

Responses were defined as improvement in two or more objective parameters, including at least one direct manifestation of myeloma (a decrease of 50% in the sum of the products of a palpable or radiographically visualized plasmacytoma, a decrease of $\geq 50\%$ of the serum "M" protein, a decrease of 50% of the urinary protein, or definite radiographic recalcification of osteolytic lesions with no new

[1]Received Oct 28, 1977; revised May 25, 1978; accepted July 10, 1978.

[2]This study was done by the Eastern Cooperative Oncology Group (ECOG) and was supported by Public Health Service grants from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. The following institutions and investigators participated in the study: University of Rochester, NY (*J. M. Bennett* and *R. F. Bakemeier;* CA–11083 and CA–11198); New York University, New York (*R. Silber;* CA–16395); Chicago Medical School, Ill (*E. Ezdinli;* CA–14144); University of Manitoba, Winnepeg, Canada (*M. Levitt*); University of Minnesota, Minneapolis (*M. Oken;* CA–20365); ECOG Statistical Center, Sidney Farber Cancer Institute, Boston, Mass (*J. C. Bailar;* CA–12721); and University of Wisconsin, Madison (*P. P. Carbone*). Other participating institutions are: Albany Medical College (CA–06594); Brookdale Hospital Center; Hahnemann Medical College; University of Ottawa and Ontario Cancer Foundation; Albert Einstein Medical College; Georgetown University (CA–02824); Jackson Memorial Hospital (CA–02822); Pennsylvania Hospital (CA–13613); Medical College of Virginia (CA–10572); and Mt. Sinai Medical Center (CA–17152).

**Reprint requests to:* John M. Bennett, MD, University of Rochester Cancer Center, 601 Elmwood Ave, PO Box 704, Rochester, NY 14642.

lesions noted) and one indirect manifestation (reduction of marrow plasmacytosis to < 5%, a hemoglobin rise of 2 g/100 ml, return of serum albumin, calcium, or BUN to normal, and improvement in PS).

Patients were considered nonevaluable if therapy was inadequate: (*a*) less than two cycles given, (*b*) early death, (*c*) inadequate data, (*d*) lost to followup, or (*e*) major protocol violations.

RESULTS

Of the 37 patients treated, 27 (including four crossovers) were evaluable and were considered to have an adequate trial. Ten patients were nonevaluable, five receiving the adriamycin arm and five receiving the bleomycin arm. There was one patient with a major protocol violation, one patient who was lost to followup after one cycle of adriamycin, and one patient who died suddenly at home 10 days after receiving 100 mg of adriamycin. The remaining seven patients all died within 21 days of initiation of therapy.

Only two patients had received no prior chemotherapy and 60% had received at least one course of radiation therapy, with no difference between those treated with adriamycin or bleomycin. In addition, most of the patients (90% receiving the adriamycin arm and 65% receiving the bleomycin arm) had responded to prior therapy. Seventy percent of the patients who received adriamycin and 63% of those who received bleomycin were in the "good-risk" category.

Of the 27 evaluable patients, there were two partial responses (one each with adriamycin and bleomycin). Additionally, in one patient receiving adriamycin, a 440% decrease in IgG occurred over a 7-week period (from 20,350 to 4580 mg/100 ml), but hypercalcemia developed and the patient died.

Slightly more than half of the patients were ambulatory (table 1), and most of these were in the "good-risk" category. There was a poorer correlation between "nonambulatory" status and "good- and poor-risk" stratification because several patients were bedridden with pain or spinal cord compression.

The overall response rate was 7.4%: 12.5% for adriamycin- and 5% for bleomycin-treated patients (table 2). Both responses occurred in "good-risk" patients (table 3). The average dose of adriamycin administered for the evaluable patients was 774 mg (104 mg for nonevaluable patients) and the average dose of bleomycin administered was 318 mg (54 mg for nonevaluable patients). If those patients who were considered nonevaluable because of failure to complete the study requirement of a minimum of 6 weeks of therapy were included, then the overall response rate was even lower, ie, 5%.

TOXICITY

Toxicity was graded according to the standard ECOG toxicity criteria (grades 0–5, with grade 5 being toxicity that caused the death of the patient). With the adriamycin regimen there were three mild (2+) and four moderate (3+) hematologic toxicities recorded. In addition, one 2+ cardiac toxicity occurred (ventricular arrhythmia). With the bleomycin regimen there were two episodes of fevers of up to 39.5°C and two patients with skin rash.

DISCUSSION

This study included the number of patients necessary to determine whether either of the agents, adriamycin or bleomycin, demonstrated activity in refractory multiple myeloma. In order to accomplish this, however, accrual of patients for about 3 years was necessary.

The major reason for the long accrual was the high percentage of nonevaluable patients, which resulted from the inability to survive for the required treatment period. The median survival of the entire group of registered patients was 32 months from diagnosis but only 4 months from the start of treatment in this program, indicating an overall dismal prognosis for this myeloma group subset.

Adriamycin has been introduced into the management of previously untreated patients with myeloma in combination with cyclophosphamide and

TABLE 1.—Comparison of PS and "good- and poor-risk" groups

ECOG PS	No. of patients	No. of patients in— "Good-risk" group	"Poor-risk" group
Ambulatory	17	14	3
Nonambulatory	15	7	8
Totals	32	21	11

TABLE 2.—Overall responses for adriamycin and bleomycin

Response	No. of patients receiving— Adriamycin	Bleomycin
Partial	1	1
No change	5	13
Progressive disease	2	5
Nonevaluable	5	5
Totals	13	24

TABLE 3.—Summary of responding patients

Patient age/sex	PS	Immuno-globulin	Previous treat-ment	Current treat-ment	Total dose (mg)	Duration of response (days)
50/F	1 ("good-risk")	IgG	Radiation	Adriamycin	374	85
81/F	3 ("good-risk")	Negative	Radiation, melphalan	Bleomycin	260	120

prednisone and with vincristine, cyclophosphamide, and prednisone by the Southwest Oncology group (6). As yet, bleomycin has not been studied in combination with other agents.

The inability to demonstrate significant activity of either of these agents may reflect the overall poor prognosis of end-stage refractory myeloma patients. Since the evaluable patients received significant doses of both agents, it is unlikely that other schedules would have made a difference.

PS, according to the ECOG criteria (table 1), has a high correlation with tumor responsiveness (7). Nevertheless, in multiple myeloma, where bone pain, severe osteoporosis, or spinal cord compression is frequent and not necessarily life-threatening, it would seem more appropriate to employ only the "good- and poor-risk" criteria for stratification purposes.

REFERENCES

1. SMITH I, and MCELWAIN T. Adriamycin in acute leukemia. Lancet 2:161, 1974.
2. MCKELVEY EM, GOTTLIEB JA, WILSON HE, ET AL. Hydroxydaunomycin (adriamycin) combination chemotherapy in malignant lymphoma. Cancer 38:1484–1493, 1976.
3. ALBERTS DS, and SALMON SE. Adriamycin (NSC–123127) in the treatment of alkylator-resistant multiple myeloma: a pilot study. Cancer Chemother Rep 59:345–350, 1975.
4. BENNETT JM, LENHARD RE, JR, EZDINLI E, ET AL. Chemotherapy of non-Hodgkin's lymphomas: Eastern Cooperative Oncology Group experience. Cancer Treat Rep 61:1079–1083, 1977.
5. Proposed guidelines for protocol studies. II. Plasma cell myeloma. Cancer Chemother Rep Part 3, vol 4(1):145–158, 1973.
6. ALEXANIAN R, SALMON S, BONNET J, ET AL. Combination therapy for multiple myeloma. Cancer 40:2765–2771, 1977.
7. ZELEN M. Importance of prognostic factors in planning therapeutic trials. *In* Cancer Therapy: Prognostic Factors and Criteria of Response (Staquet MJ, ed). New York, Raven Press, 1975, pp 1–6.

Phase I Study of N^6-Benzyladenosine-5′-monophosphate [1]

Brief Reports and Preliminary Communications

Raphael Catane,* Jerome H. Kaufman, Freda A. Nime, James T. Evans, and Arnold Mittelman [2,3]

The riboside of N^6-benzyladenine has shown antitumor effects in animal systems (1,2) and in a limited clinical trial (3). The low solubility of this compound limited it to oral administration. The present availability of the 5′-monophosphate salt (fig 1) enabled us to perform a new phase I study using the iv route.

METHODS AND PATIENTS

The toxic dose of oral N^6-benzyladenosine-5′-monophosphate (N^6BAR5P) in the previous study (3) was 40 mg/kg for 3 days. We therefore started with one half of that dose (20 mg/kg/day) and this was given for 3 days iv. N^6BAR5P was infused over 2–3 hours because hypotension was reported in animal studies when the drug was given rapidly (4).

Fourteen patients with advanced carcinomas and sarcomas refractory to all conventional chemotherapy were treated with N^6BAR5P (supplied by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md). All patients had given voluntary informed consent prior to receiving the drug. Seven patients received 20 mg/kg/day, four received an escalated dose of 30 mg/kg/day, and three received 40 mg/kg/day. In the absence of disease progression and significant toxicity the patient was re-treated after 3 weeks with the same dose of N^6BAR5P; no patient had a dose escalation.

RESULTS AND DISCUSSION

The dose-limiting toxicity was hepatic (table 1). The onset of liver toxicity was seen within 24 hours and was maximal on the third day of treatment; a liver biopsy at that time showed diffuse small droplet steatosis and rare foci of necrosis with inflammatory reaction (fig 2). In every instance, the liver abnormalities (elevated bilirubin and enzyme levels) returned to normal within 1 week. Two patients who had hepatic impairment related to the drug were re-treated with the low dose (20 mg/kg/day × 3 days), and only mild abnormality in liver function tests occurred (fig 3).

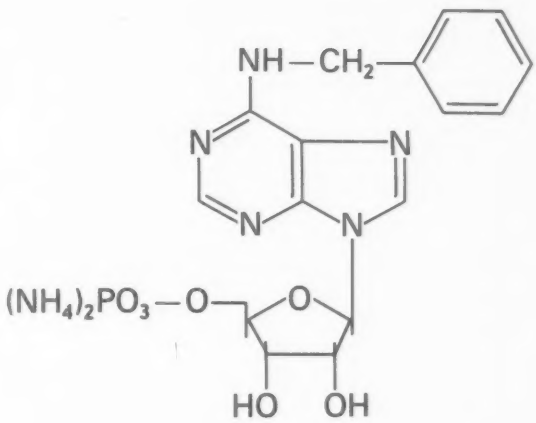

FIGURE 1.—Structure of the ammonium salt of N^6BAR5P.

A 75-year-old patient with a previous history of coronary heart disease developed nodal tachycardia while receiving the drug which reverted to normal rhythm when the infusion rate was lowered. No orthostatic hypotension was noted. None of the patients developed myelotoxicity to any degree, even after repeated doses. Seven patients received repeated courses of treatment and three patients had > 200 mg/kg of the drug over a 2-month period without any signs of cumulative toxicity. No objective responses were seen in this group of solid tumor patients, all of whom had had extensive prior chemotherapy.

The drug dose for a phase II study should be between 20 and 30 mg/kg (or 1 g/m^2) daily for 3 consecutive days and repeated every 3 weeks. Pa-

[1]Received Feb 1, 1978; revised June 19, 1978; accepted July 13, 1978.

[2]Departments of Surgical Developmental Oncology and Pathology, Roswell Park Memorial Institute, Buffalo, NY.

[3]We thank Mr. William T. Soper for reviewing the manuscript.

**Reprint requests to:* Raphael Catane, MD, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bldg 37, Rm 6E12, National Institutes of Health, Bethesda, Md 20014.

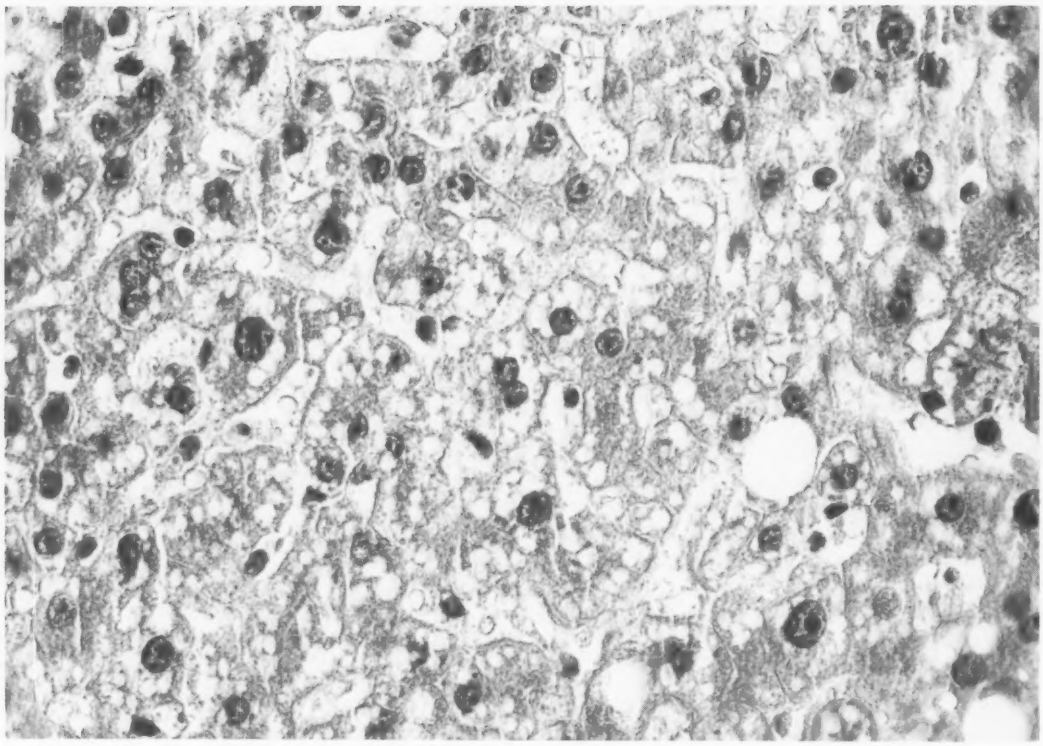

FIGURE 2.—Liver biopsy on the 3rd day of N^6BAR5P administration. Liver shows diffuse marked fatty metamorphosis in the form of fine droplets of fat packed in the cytoplasm of the liver cell, similar to that seen in tetracycline toxicity (5) (hematoxylin and eosin stain, × 680). In addition, rare foci of liver cell necrosis with surrounding inflammatory cells were present (*not shown*).

tients with a history of myocardial disease should be monitored during drug administration, and special attention should be given to patients with pretreatment liver abnormalities.

This purine analog is an example of an anticancer drug which does not have myelosuppression as its dose-limiting toxic effect. Drugs similar in this characteristic include, among others, vincristine, bleomycin, streptozotocin, L-asparaginase, and the recently introduced hycanthone and maytansine. Such drugs require use of special precautions, since routine blood cell counts will not herald toxicity, and they are all potentially useful for combination with myelotoxic agents.

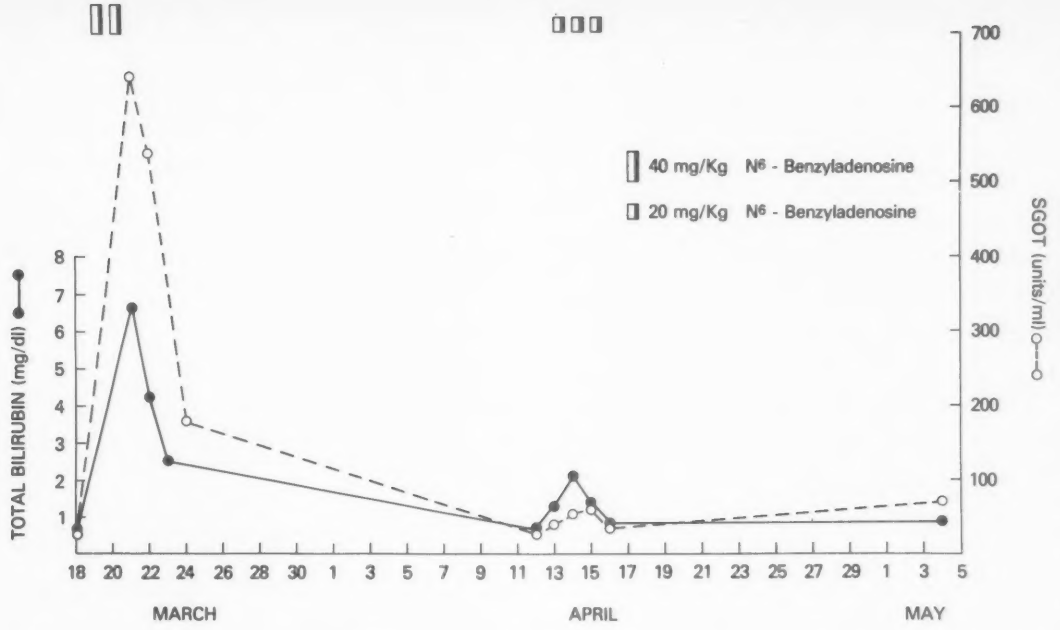

FIGURE 3.—Liver function tests during N^6BAR5P administration. This is a 68-yr-old white man with colon cancer metastatic to the lung (no known liver metastases). He had marked liver abnormalities after 2 days of 40 mg/kg of N^6BAR5P but only minor abnormalities after a subsequent 3-day course of 20 mg/kg/day.

TABLE 1.—Hepatic toxicity after 1 course of N^6BAR5P

	No. of patients			
		Degree of toxicity*		
Dosage	Total	None	Mild	Moderate
20 mg/kg/day × 3 days	7	5	2	0
30 mg/kg/day × 3 days	4	2	2	0
40 mg/kg/day × 3 days	3†	0	0	3

*Toxic effects included elevation of bilirubin and liver enzyme levels. For patients who had normal values prior to N^6BAR5P therapy, the range of elevation for "mild" toxicity was bilirubin up to 3.0 mg/dl, SGOT up to 150 units/ml, and alkaline phosphatase up to 250 IU/ml (normal range in our laboratory was up to 1.0 mg/dl, 40 units/ml, and 80 IU/ml, respectively); "moderate" toxicity was defined as elevation of the bilirubin and enzymes to higher levels, with prompt recovery.

†2 of these patients were treated for only 2 days because of liver toxicity (see fig 3).

REFERENCES

1. FLYSHER MH, BLOCH A, HAKALA MT, ET AL. Synthesis and biological activity of some new N^6-substituted purine nucleosides. J Med Chem 12:1056–1061, 1969.
2. RUSTUM Y, AL-HUSSAINY T, SIMPSON C, ET AL. Antiproliferative activity of N^6-benzyladenosine (BAR) in rats. Proc Am Assoc Cancer Res 14:60, 1973.
3. MITTELMAN A, EVANS JT, and CHHEDA GB. Cytokinins as chemotherapeutic agents. Ann NY Acad Sci 255:225–234, 1975.
4. AL-HUSSAINY TM. Studies on *N*6-benzyladenosine: selective toxicity and mode of action. Doctoral thesis. State University of New York at Buffalo, 1973.
5. POPPER H. Drug induced injury. *In* The Liver (Gall ED, and Mostofi FK, eds). Baltimore, Williams and Wilkins Co, 1973, p 182.

Adriamycin Therapy for Advanced Ovarian Carcinoma Recurrent After Chemotherapy [1]

Brief Reports and Preliminary Communications

Susan Molloy Hubbard,[2,*] Peter Barkes,[2,3] and Robert C. Young [2]

Adriamycin is an antitumor antibiotic which has demonstrated significant activity in a broad spectrum of human cancer, including gynecologic malignancies (1). This report summarizes the results seen with adriamycin given as a single agent to patients with advanced ovarian cancer who had developed recurrent disease or disease progression during or after treatment with systemic chemotherapy and, in several patients, with concurrent pelvic irradiation.

MATERIALS AND METHODS

Eighteen patients with advanced ovarian carcinoma (FIGO stage III or FIGO stage IV disease) who had previously received chemotherapy and/or local radiotherapy at the Medicine Branch, National Cancer Institute, from August 1971 to July 1976, have received adriamycin as single-agent therapy for disease progression. All patients were considered evaluable for response and toxicity.

All of the 18 patients had ovarian carcinoma of epithelial origin. The median age was 50 years (range, 35–76 years). The diagnosis of ovarian cancer in all patients was made by pathologic examination of tumor tissue obtained during exploratory laparotomy. The histologic cell type was serous carcinoma in 12 patients and poorly differentiated or undifferentiated carcinoma in six patients.

Initial chemotherapy consisted of a single alkylating agent in six patients. Five of these patients received oral melphalan (0.2 mg/kg/day × 5 days, every 4–6 weeks), and one patient received daily oral chlorambucil (0.2 mg/kg/day continuously). Twelve patients were treated with an intensive combination chemotherapy regimen: hexamethylmelamine and cyclophosphamide, 150 mg orally daily × 14 days; 5-fluorouracil (5–FU), 600 mg/m² on Days 1 and 8; and methotrexate, 40 mg/m² on Days 1 and 8 (2).

The responses of patients to initial chemotherapy can be summarized as follows: only two of 18 patients obtained a complete remission (CR), ten obtained a partial remission (PR), and six had no response to therapy. Twelve of 18 patients received one or more chemotherapeutic agents prior to treatment with adriamycin. Three of these patients had previously received local pelvic irradiation. At the time adriamycin therapy was initiated, ten of 15 patients originally classified as stage III by FIGO criteria had progressed to stage IV disease on the basis of dissemination beyond the abdominal cavity (3).

Hematopoietic insufficiency due to extensive chemotherapy was encountered in 15 of the 18 patients prior to treatment with adriamycin. This recurrent pancytopenia necessitated a reduction in adriamycin dosage from 60 mg/m² every 3 weeks to 30–40 mg/m² every 3 weeks. The initial dose chosen for each patient was repeated at 3-week intervals unless progressive disease was evident, the wbc count was $< 3000/mm^3$, or the platelet count was $< 100{,}000/mm^3$. If no significant hematologic depression was encountered, the dose of adriamycin was increased to maximize potential antitumor effect.

EVALUATION OF RESPONSE

The following criteria for evaluating the magnitude of response to therapy were used. A CR was defined as the regression of all evidence of ovarian cancer for at least 1 month and no residual microscopic tumor documented during restaging peritoneoscopy and/or "second-look" laparotomy. A PR was indicated by a $\geq 50\%$ regression of all measurable tumor for a minimum of 1 month. An objective response was a 25%–50% regression of measurable tumor for at least 1 month. No response was a $< 25\%$ regression of measurable tumor or regression of some tumor without concomitant regression in other areas. Disease progression was defined as any increase in measurable tumor or the appearance of new lesions during treatment.

[1] Received May 4, 1978; revised June 21, 1978; accepted June 29, 1978.

[2] Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

[3] *Present address:* Royal Marsden Institute of Cancer Research, Department of Nursing Studies, London, England.

* *Reprint requests to:* Susan Molloy Hubbard, RN, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 12N226, National Institutes of Health, Bethesda, Md 20014.

RESULTS

Single-agent adriamycin therapy produced no responses of clinical benefit in this patient population which had received extensive prior therapy (table 1). One patient with a large abdominal mass obtained a 40% reduction in measurable abdominal tumor lasting 2 months, but did not experience objective or subjective improvement in performance status. The median survival in this group of patients following initiation of adriamycin therapy was 3 months (range, 0.3–33+ months). Only one patient remains alive after adriamycin therapy with recurrent disease which is being controlled with *cis*-dichlorodiammineplatinum(II).

Toxic effects from adriamycin included alopecia and mild to moderate nausea and vomiting in virtually all patients. No mucositis, cardiotoxicity, or hepatotoxicity was encountered. Hematologic toxic effects are summarized in table 2. Forty-four doses of adriamycin were administered to these 18 patients; the total doses of adriamycin ranged from 30 to 405 mg/m² (median, 65 mg/m²).

DISCUSSION

Women with FIGO stage III and IV ovarian carcinoma are known to be responsive to a variety of chemotherapeutic agents. Response rates of 35%–65% can be obtained with most alkylating agents with 5%–15% of all patients continuing to respond 2 years after the initiation of therapy (4). The anti-

TABLE 1.—Characteristics of patients receiving adriamycin

Patient No.	Prior therapy*	Disease stage at initiation of adriamycin	Total adriamycin dose (mg/m²)	No. of courses given	Response type
1	L-PAM, Hexa-CAF, and local radiotherapy	IV	30	1	None
2	L-PAM, cyclophosphamide, and hexamethylmelamine	IV	160	4	None
3	Hexa-CAF	IV	90	2	None
4	Local radiotherapy, Hexa-CAF, and L-PAM	IV	60	2	None
5	Hexa-CAF, cyclophosphamide, and cytembena	III	405	10	None
6	Hexa-CAF	III	90	3	None
7	Hexa-CAF	IV	60	1	None
8	Hexa-CAF and procarbazine	III	30	1	None
9	Hexa-CAF	IV	60	2	None
10	Hexa-CAF	IV	45	1	None
11	L-PAM, local radiotherapy, 5-FU, hexamethylmelamine, and methotrexate	IV	150	3	None
12	L-PAM, hexamethylmelamine, and 5-FU + hexamethylmelamine	IV	180	3	None
13	Hexa-CAF	IV	85	2	None
14	Hexa-CAF and cytembena	IV	45	1	None
15	Hexa-CAF and local radiotherapy	IV	60	1	None
16	Hexa-CAF, L-PAM, *cis*-dichlorodiammineplatinum(II), and cytembena	IV	45	1	None
17	Chlorambucil, hexamethylmelamine, methotrexate, and local radiotherapy	III	70	2	None
18	L-PAM and hexamethylmelamine	III	180	4	Objective

*L-PAM = melphalan; Hexa-CAF = hexamethylmelamine, cyclophosphamide, 5-FU, and methotrexate.

TABLE 2.—Hematologic toxic effects of adriamycin (total: 18 patients)

Hematologic toxic effects	No. of patients (%)
Leukopenia (wbcs/mm³)	
> 3000	6 (33)
2000–2999	4 (22)
1000–1999	6 (33)
< 1000	2 (11)
Thrombocytopenia (platelets/mm³)	
> 150,000	8 (44)
50,000–150,000	5 (28)
< 50,000	5 (28)

metabolites, 5–FU and methotrexate, can produce objective responses in approximately 30% of patients (5). In the recent past, adriamycin and hexamethylmelamine have been shown to have activity in advanced ovarian cancer, both of which produce objective response rates of 40% when used as primary therapy (6). Although a number of agents are active against advanced ovarian cancer, many patients do not respond to their initial chemotherapy and the development of drug resistance is common. The choice of subsequent treatment for patients who develop progressive disease while receiving chemotherapy is a formidable problem. Only a few published clinical studies report responses in ovarian cancer patients to a second or third trial of chemotherapy. As in this study, response rates to a second or third trial of chemotherapy are characteristically low. Agents which have been studied in this situation includes 5–FU, high-dose methotrexate, high-dose cyclophosphamide, triethylenethiophosphoramide, hexamethylmelamine, and *cis*-dichlorodiammineplatinum(II) (7–11).

Two studies, one by de Palo et al (12) and one by Stanhope et al (7) have reported results with single-agent adriamycin used as initial chemotherapy for advanced ovarian cancer and as the second agent in patients whose disease had progressed or recurred during treatment with other drugs. de Palo and co-workers reported a 50% CR and PR rate (seven of 14 patients) to adriamycin in untreated patients (12). Stanhope et al (7) reported a 33% objective response rate in 12 untreated patients who received adriamycin. In 12 patients given adriamycin for disease progression, de Palo et al reported one response in six patients previously treated with unspecified agents and four PRs in six evaluable patients who had previously received melphalan only (12). Stanhope and co-workers (7) reported no responses in 27 patients given adriamycin as a second therapy. Bolis et al (13) have studied adriamycin in patients who had been previously treated with low-dose cyclophosphamide; only three of 38 patients obtained a response to adriamycin therapy. All patients were evaluated after three cycles of adriamycin given at a dose of 60 mg/m² every 3 weeks.

Our results in 18 patients show no clinically useful response to adriamycin in patients treated extensively with other chemotherapeutic agents prior to adriamycin. These results are consistent with other investigators testing the agent in similar patients. These results suggest that the low overall response rate of 14% (20 of 138 patients) reported by Slavik (14) may reflect the fact that single-agent adriamycin is of little therapeutic value for patients who have undergone extensive prior therapy.

REFERENCES

1. BLUM RH, and CARTER SK. Adriamycin: a new anticancer drug with significant clinical activity. Ann Intern Med 80:249–259, 1974.
2. YOUNG RC, CHABNER BA, HUBBARD SP, ET AL. Advanced ovarian adenocarcinoma: melphalan versus combination chemotherapy (Hexa-CAF). Proc Am Assoc Cancer Res and ASCO 19:393, 1978.
3. DAY TG, and SMITH JP. Diagnosis and staging of ovarian cancer. Semin Oncol 2:217–222, 1975.
4. BAGLEY CM, YOUNG RC, and CANELLOS GP. Treatment of ovarian carcinoma; possibilities for progress. N Engl J Med 287:856–862, 1972.
5. YOUNG RC. Chemotherapy of ovarian cancer: past and present. Semin Oncol 2:267–276, 1975.
6. DEVITA VT, WASSERMAN TH, YOUNG RC, ET AL. Perspectives on research in gynecologic oncology: treatment protocols. Cancer 38:509–525, 1976.
7. STANHOPE RC, SMITH JP, and RUTLEDGE F. Second trial drugs in ovarian cancer. Gynecol Oncol 5:52–58, 1977.
8. BARLOW JJ, and PIVER MS. Methotrexate (NSC–740) with citrovorum factor (NSC–3590) rescue, alone and in combination with cyclophosphamide (NSC–26271), in ovarian cancer. Cancer Treat Rep 60:527–533, 1976.
9. PIVER MS, BARLOW JJ, and CHUNG WS. High-dose cyclophosphamide (NSC–26271) for recurrent or progressive ovarian adenocarcinoma. Cancer Chemother Rep 59:1157–1158, 1975.
10. TWIGGS LB, and MORROW CP. Treatment of advanced ovarian carcinoma with high-dose cyclophosphamide after failure on melphalan. Cancer Treat Rep 61:1369–1371, 1977.
11. WILTSHAW E, and KRONER T. Phase II study of *cis*-dichlorodiammineplatinum(II) (NSC–119875) in advanced adenocarcinoma of the ovary. Cancer Treat Rep 60:55–60, 1976.
12. DE PALO GM, DE LENA M, DI RE F, ET AL. Melphalan versus adriamycin in the treatment of advanced carcinoma of the ovary. Surg Gynecol Obstet 141:899–902, 1975.
13. BOLIS G, D'INCALCI M, GRAMELLINI F, ET AL. Letter: Adriamycin in ovarian cancer patients resistant to cyclophosphamide. Eur J Cancer. In press.
14. SLAVIK M. Adriamycin (NSC–123127) activity in genitourinary and gynecologic malignancies. Cancer Chemother Rep Part 3, vol 6(2):297–303, 1975.

Chemo-Hormonal Therapy for Metastatic Renal Cell Carcinoma With Adriamycin, Hydroxyurea, Vinblastine, and Medroxyprogesterone Acetate [1]

Brief Reports and Preliminary Communications

S. B. Katakkar[2,*] and C. R. Franks[3,4]

SUMMARY

In a phase II study, eight patients with metastatic renal cell carcinoma have been treated with a combination of chemotherapy and hormonal therapy using adriamycin, hydroxyurea, vinblastine, and medroxyprogesterone acetate. Five patients have responded, including one with complete response, one with subjective improvement, and three with partial responses. The median survival was 4 months with a range of 4–11 months. With the exception of mild alopecia and nausea, toxicity was minimal; no significant hematologic toxicity was noted. The initial results of this study suggest that chemo-hormonal therapy plays a significant role in the management of renal cell carcinoma.

[Cancer Treat Rep 62:1379–1380, 1978]

The prognosis in metastatic renal cell carcinoma is very poor, with survival rates of 5% and 0 at 5 and 10 years respectively. Spontaneous regression of the disease has been reported, but this occurs in < 1% of the cases (1). A combined chemo-hormonal therapeutic approach has been used before (2); however, the results have not been encouraging. Vinblastine is the only agent shown to be effective (3); other chemotherapeutic agents such as adriamycin (4) and hydroxyurea (5) have shown some activity, although they are not comparable to vinblastine alone. In Saskatchewan, we have used combined chemo-hormonal therapy incorporating agents such as vinblastine, adriamycin, hydroxyurea, and medroxyprogesterone acetate. The present study is a phase II trial, and if a meaningful response is obtained, further patients will be randomized to chemotherapy alone or chemo-hormonal therapy.

MATERIALS AND METHODS

Eight patients (seven with histopathologic diagnosis of metastatic renal cell carcinoma and one with classic angiographic findings of renal cell carcinoma) have been treated. Prior to the start of therapy, all patients were evaluated for the extent of disease with full biochemical, hematologic (excluding bone marrow), and metastatic investigations including chest roentgenograms (posteroanterior and lateral), lung tomograms if metastatic disease was noted on routine chest roentgenogram, and bone and liver scans. The chemo-hormonal protocol used included adriamycin (25 mg/m² iv, Days 0 and 7), vinblastine (5 mg/m² iv, Days 0 and 7), hydroxyurea (10 mg/kg/day, Days 0–5), and medroxyprogesterone acetate (200 mg im, Days 21, 24, and 27). The chemo-hormonal therapy was repeated every 4 weeks.

The response to therapy was evaluated both subjectively and objectively. An objective response was either a complete remission, with a total disappearance of all metastatic disease, or a partial remission, with a > 50% decrease in the size of one or more measurable lesions, in the absence of progressive disease. When concomitant radiotherapy was provided, the area to which radiation was given was not included for evaluation. A complete physical examination was performed prior to each course of chemotherapy. Cbc counts were obtained prior to each dose of chemotherapy and an electrocardiogram and chest roentgenogram were done on Day 0 of each course of chemotherapy.

RESULTS

To date, eight patients (seven men and one

[1] Received Mar 17, 1978; revised June 19, 1978; accepted June 27, 1978.

[2] Allan Blair Memorial Clinic, Saskatchewan Cancer Commission, Regina, Canada.

[3] University Hospital, Saskatoon Cancer Clinic, Saskatchewan, Canada.

[4] We thank the medical staff of the Saskatchewan Cancer Commission for their assistance and permission to furnish the data, Dr. H. I. Pierce for his comments, and Linde Konkel for her typographical assistance.

*Reprint requests to: S. B. Katakkar, MD, Medical Oncology Service, Madigan Army Medical Center, Tacoma, Wash 98431.

TABLE 1.—Patient data

Patient No./age/sex	Metastatic site	Histopathology of primary	Response	Duration (mos)	Status	No. of courses
1/53/M	Lung	Clear cell carcinoma	Partial	7	Alive	8
2/71/M	Abdominal carcinoma	Clear cell carcinoma	Partial, static	11	Alive	10
3/64/M	Lung	No histopathology	None	4	Alive	4 (chemotherapy stopped)
4/48/M	Lung	Adenocarcinoma	Complete	4	Alive	4
5/69/M	Both femora	Clear cell carcinoma	Partial, subjective improvement	4	Alive	4
6/63/M	Abdominal carcinomatosis	Papillary adenocarcinoma	Partial	4	Alive	4
7/44/F	Regional lymph nodes	Clear cell carcinoma	None	4	Alive	3 (chemotherapy stopped)
8/69/M	Multiple bones, lung	Clear cell carcinoma	None	2	Dead	2

woman ranging in age from 44 to 71 years) have been studied. The clinical data and results are summarized in table 1. Three of the eight patients failed to respond. Two of these patients had three courses of chemotherapy and one had progressive disease after two courses of chemotherapy (this patient has died). Five patients showed response; one patient with lung metastasis has shown complete response after four courses of chemotherapy. Of the remaining four patients, one had subjective response with the disappearance of constitutional symptoms of fatigue and excessive sweating and an increase in his weight. In this instance, there is no evidence of progressive disease after four courses of chemo-hormonal therapy. The patient did receive radiation therapy to both femora, but these sites are not included in the objective evaluation. Of the other three patients, two had abdominal carcinomatosis (patients 2 and 6). A repeat laparotomy in one patient revealed a complete disappearance of peritoneal secondaries. The previously inoperable left kidney tumor was removed and was found to be necrotic after three courses of chemotherapy. The other patient with abdominal carcinomatosis has done well for 11 months; the left renal tumor is still palpable but unchanged in size. Patient 1 had shown almost complete response of the metastatic lung disease after eight courses of chemotherapy.

With the exception of some alopecia and slight nausea, none of the patients has experienced overt subjective or objective toxicity. Full chemotherapeutic dosages were administered since there was no hematologic toxicity. There was no evidence of cardiotoxicity, although none of the patients reached the cardiotoxic levels of adriamycin.

In view of these early but encouraging results, the above chemo-hormonal therapy seems to have merit and further studies are warranted.

REFERENCES

1. BLOOM HJG. Adjuvant therapy for adenocarcinoma of the kidney: present position and prospects. Br J Urol 45:237–257, 1973.
2. HAHN RG, and BRODOVSKY H. Methyl CCNU, Velban and Depo-Provera treatment trials in advanced renal cancer. Proc Am Assoc Cancer Res and ASCO 17:246, 1976.
3. HRUSHESKY WJ, and MURPHY GP. Current status of the therapy of advanced renal cell carcinoma. J Surg Oncol 9:277–288, 1977.
4. CARTER SK, and WASSERMAN TH. Chemotherapy of urological cancer. Cancer 36:729–747, 1975.
5. NEVINNY HB, and HALL TC. Chemotherapy with hydroxyurea in renal cell carcinoma. J Clin Pharmacol 8:352–359, 1968.

Age-Related Adriamycin Cardiotoxicity in Children [1,2]

Charles B. Pratt,* J. Laurence Ransom, and William E. Evans [3]

Brief Reports and Preliminary Communications

The recent report by Smith et al (1) regarding the high incidence of cardiotoxicity in children treated with adriamycin and DTIC may have implications other than the possible additive influence of DTIC or vincristine and VM-26 on the clinical expression of adriamycin cardiotoxicity. We have reviewed the cumulative adriamycin dose after which cardiotoxic effects developed in 21 children reported in the literature (1–8) and in four children observed at this institution. This review suggests that the risk of cardiotoxicity may occur at lower dose levels in younger children. Cyclophosphamide (9–11), other chemotherapeutic agents (12), and cardiopulmonary-mediastinal irradiation (3,8,13–15) may be additional contributing factors which have an additive influence on the dose level at which adriamycin cardiotoxicity develops, irrespective of age.

Patients known to have adriamycin cardiotoxic reactions were divided by age groups: 2–5 years, 6–10 years, and 11–20 years (table 1); these patients developed cardiotoxic effects at median adriamycin doses of 480 mg/m^2 (range, 350–590 mg/m^2), 480 mg/m^2 (range, 400–1155 mg/m^2), and 810 mg/m^2 (range, 400–1695 mg/m^2), respectively. In the 13 patients known to have received cardiopulmonary-mediastinal irradiation, the median cumulative dose of adriamycin associated with cardiotoxicity was 470 mg/m^2 (range, 350–720 mg/m^2), compared to a median cumulative dose of 550 mg/m^2 (range, 360–1695 mg/m^2) in the ten patients who did not receive similar irradiation. Cardiotoxicity was not reported in any children > 10 years of age who had not received cardiac irradiation and in whom the total adriamycin dose was ≤ 550 mg/m^2, but it was reported in five children ≤ 10 years of age who also did not receive cardiac irradiation and received adriamycin at a total dose ≤ 550 mg/m^2.

The duration of administration of the total dose of adriamycin is shown in table 1 for patients completely described by previous investigators. The median duration of drug administration was calculated for each of the three age groups and was 7.5 months (range, 5–13 months) for the 2–5-year-old group, 8 months (range, 4.5–15 months) for the 6–10-year-old group, and 18 months (range, 9–29 months) for the 11–20-year-old group. The dose of adriamycin per square meter per day was calculated for the patients for whom information regarding cumulative dose and duration of administration of the drug was available. The 2–5-year-old patients received 1.57–2.34 mg/m^2/day of adriamycin (median 1.89 and mean 1.95 mg/m^2/day); 6–10-year-old patients received 1.2–3.13 mg/m^2/day (median 1.95 and mean 2.10 mg/m^2/day); and 11–20-year-old patients received 0.7–3.4 mg/m^2/day (median 2.19 and mean 2.03 mg/m^2/day). Since the intensity of adriamycin administration (mg/m^2/day) was similar for children of all ages, the difference in duration of adriamycin administration (median, 8 months for children < 10 years versus 18 months for children > 10 years) may have been the result of younger children developing cardiotoxic effects at a lower total dose. Many of the 2–10-year-old patients received adriamycin as a single agent, whereas older patients frequently received other agents concurrently with adriamycin.

The relation of delivery of cardiopulmonary-mediastinal irradiation to administration of adriamycin was also examined as a factor which might influence the development of adriamycin cardiotoxicity (table 1). Eight children received radiation therapy prior to adriamycin, and five received concurrent adriamycin-cardiopulmonary or mediastinal irradiation. One patient who had received prior pulmonary irradiation received additional pulmonary-mediastinal irradiation concurrently with adriamycin. No patient received adriamycin followed by radiotherapy. Mediastinal irradiation preceded by 1 year the administration of adriamycin to the patient with Hodgkin's disease (8).

It is of interest that 11 of the 25 patients who developed cardiomyopathy had a diagnosis of Ewing's sarcoma, and that six of the 11 received irradiation to the chest. The evaluation of any

[1]Received Mar 21, 1978; revised June 9, 1978; accepted June 15, 1978.

[2]Supported by Public Health Service grants CA-21765 (National Cancer Institute) and RR-05584 (Division of Research Resources) from the National Institutes of Health, Department of Health, Education, and Welfare, by American Cancer Society Clinical Fellowship No. 4015, and by ALSAC.

[3]St. Jude Children's Research Hospital, Memphis, Tenn.

*Reprint requests to: Charles B. Pratt, MD, St. Jude Children's Research Hospital, 332 N Lauderdale, PO Box 318, Memphis, Tenn 38101.

TABLE 1.—Adriamycin cardiotoxic effects in children

Age (yrs)/ sex	Diagnosis	Total adriamycin dose (mg/m²)/ duration	Cardiac irradiation (rads)	Other agents*	Cause of death	Ref No.
			2–5 yrs			
5/F	Wilms' tumor	350/5 mos	1260 to bilateral lungs† 1500 to left lung‡ 1200 to right mediastinum‡	VCR† Actinomycin D†	Cardiac	2
4/?	Ewing's sarcoma	360/6 mos	None	VCR‡ DTIC‡	Living	1
5/F	Ewing's sarcoma	422/6 mos	1300 to right lung‡ 1200 to left lung‡	VCR Cyclophosphamide (6140 mg/m²)§	Cardiac	SJCRH¶
3/F	Wilms' tumor	450/not stated	Mediastinum†	VCR† Actinomycin D†	Tumor	5
5/?	Ewing's sarcoma	480/8 mos	None	VM-26‡ DTIC‡	Cardiac	1
4/M	Osteosarcoma	483/9 mos	None	HDMTX‡ Cyclophosphamide (3442 mg/m²)‡	Cardiac	SJCRH
3/F	Wilms' tumor	500/not stated	Mediastinum†	VCR† Actinomycin D†	Tumor	5
3/?	?	200–575/not stated	?	None	Cardiac	2,4
2/F	Embryonal carcinoma	590/13 mos	1200 to right mediastinum‡	VCR‡ Actinomycin D‡ Cyclophosphamide‡	Living	2
			6–10 yrs			
8/M	Wilms' tumor	400/4.5 mos	1200 to right lung† 4250 to right lung† 2558 to right hilus,† mediastinum	VCR† Actinomycin D†	Living	2
8/?	Ewing's sarcoma (rib)	420/7.5 mos	4000 to chest‡	VM-26‡ DTIC‡	Cardiac	1
9/F	Ewing's sarcoma	470/5 mos	2250 to whole lung‡		Cardiac	8
6/F	Ewing's sarcoma	473/7 mos	1500 to left hemithorax† 7000 to right scapula†	VCR† Cyclophosphamide (15,244 mg/m²)†	Cardiac	SJCRH
6/?	Ewing's sarcoma	480/9 mos	None	VCR‡ DTIC‡	Cardiac	1
6/M	Neuroblastoma	540/15 mos	None	Cyclophosphamide‡ VCR‡ VM-26§ Ara-C§	Tumor	SJCRH

10/M	Osteosarcoma	675/11 mos	None	VCR‡ Actinomycin D‡ Cyclophosphamide‡	Cardiac	3
7/M	Ewing's sarcoma	720/15 mos	2100 to mediastinum†	VCR‡ Actinomycin D‡ Cyclophosphamide‡	Living	3
10/?	?	409–1155/not stated	?	None	Living	2,4
			11–20 yrs			
11/F	Ewing's sarcoma	400/not stated	3400 to right heart‡	?	Living	8
11/F	Osteosarcoma	600/9 mos	1400 to right lung†	VCR‡ Actinomycin D‡ Cyclophosphamide‡	Tumor	3
11/F	Hodgkin's lymphoma	120 (daunorubicin), 495 (adriamycin)/ 29 mos	3500 to mediastinum†	VCR‡ Actinomycin D‡ Cyclophosphamide‡	Living	3
11/F	Teratocarcinoma	810/22 mos	None	VCR‡ Actinomycin D‡ Cyclophosphamide‡	Cardiac	3
14/M	Ewing's sarcoma	905/14 mos	None	VCR‡ Actinomycin D‡ Cyclophosphamide‡	Living	3
17/M	Osteosarcoma	1125/11 mos	None	None	Cardiac	6,7
12/M	Ewing's sarcoma	1695/22 mos	None	VCR‡ Actinomycin D‡ Cyclophosphamide‡	Tumor	3

*VCR = vincristine, HDMTX = high-dose methotrexate, and Ara-C = cytosine arabinoside.
†Prior to adriamycin.
‡Concurrent with adriamycin.
§Following adriamycin.
¶SJCRH = St. Jude Children's Research Hospital.

relationship to dose of other agents, eg, cyclophosphamide, could not be made in these patients because of lack of information on the administration and/or cumulative dose of the agent.

Following the recognition of adriamycin (or daunorubicin)-associated cardiomyopathy, oncologists have generally followed the guidelines provided by Lefrak et al (16) and restricted the cumulative dose to < 550 mg/m^2. The observations of Rinehart and co-workers (17) and Ramos et al (18) that prolongation of systolic time intervals (PEP/LVET) or changes in thickness of the left ventricular wall (18,19) correlate with adriamycin cardiotoxicity may provide additional guidelines regarding continuation of adriamycin administration (20). Endomyocardial biopsy may be a still more sensitive index for determination of frank cardiomyopathy (15).

The incidence of adriamycin cardiotoxicity was reported to be only 0.27% in 399 adults receiving < 550 mg/m^2 (16). Other investigators (7,21,22) have also reported a low incidence of cardiotoxicity when total adriamycin doses were limited to < 550 mg/m^2. The incidence of cardiotoxic effects in children could not be determined from the data summarized in this report since the number of patients at risk was not known. However, adriamycin cardiotoxic effects have developed in four of 36 children who received 400–550 mg/m^2 of adriamycin at our institution, in four of ten children who received 360–540 mg/m^2 as reported by Smith et al (1), and in two of 13 children who received 450–500 mg/m^2 as reported by Cortes et al (7). It is important to note that eight of the 14 children summarized in this report who developed cardiotoxic effects at a dose ≤ 550 mg/m^2 had also received irradiation to either the lung, heart, or mediastinum, and that no child > 10 years of age developed cardiomyopathy at a dose of < 550 mg/m^2 in the absence of irradiation. A previous review of 5613 patients treated with daunorubicin suggested that children may be more susceptible to daunorubicin-induced cardiomyopathy than adults (23). A similar analysis of risk factors has indicated that schedule of administration of adriamycin may be an even more significant determinant, with patients who receive low weekly doses having a significantly smaller risk of developing adriamycin-induced congestive heart failure (14,24). More accurate assessment of adriamycin cardiotoxic reactions in children will require additional data obtained prospectively from pediatric institutions and cooperative groups treating large numbers of patients.

REFERENCES

1. Smith PJ, Ekert H, Waters KD, et al. Letter: High incidence of cardiomyopathy in children treated with adriamycin and DTIC in combination chemotherapy. Cancer Treat Rep 61:1736–1738, 1977.
2. Prout MN, Richards MJS, Chung KJ, et al. Adriamycin cardiotoxicity in children. Case reports, literature review and risk factors. Cancer 39:62–65, 1977.
3. Gilladoga AC, Manuel C, Tan CC, et al. Cardiotoxicity of adriamycin (NSC-123127) in children. Cancer Chemother Rep Part 3, vol 6(2):209–214, 1975.
4. Bonadonna G, Beretta G, Tancini G, et al. Adriamycin (NSC-123127) studies at the Istituto Nazionale Tumori, Milan. Cancer Chemother Rep Part 3, vol 6(2):231–245, 1975.
5. Wang JJ, Holland JF, and Sinks LF. Phase II study of adriamycin (NSC-123127) in childhood solid tumors. Cancer Chemother Rep Part 3, vol 6(2):267–270, 1975.
6. Cortes EP, Holland JF, Wang JJ, et al. Adriamycin (NSC-123127) in 87 patients with osteosarcoma. Cancer Chemother Rep Part 3, vol 6(2):305–313, 1975.
7. Cortes EP, Lutman G, Wanka J, et al. Adriamycin (NSC-123127) cardiotoxicity: a clinicopathologic correlation. Cancer Chemother Rep Part 3, vol 6(2):215–225,1975.
8. Merrill J, Greco FA, Zimbler H, et al. Adriamycin and radiation: synergistic cardiotoxicity. Ann Intern Med 82:122–123, 1975.
9. O'Connell TX, and Berenbaum MC. Cardiac and pulmonary effects of high doses of cyclophosphamide and iphosphamide. Cancer Res 34:1586–1591, 1974.
10. Mullins GM, Anderson PN, and Santos GW. High dose cyclophosphamide therapy in solid tumors. Therapeutic, toxic and immunosuppressive effects. Cancer 36:1950–1958, 1975.
11. Slavin RE, Millan JC, and Mullins GM. Pathology of high dose intermittent cyclophosphamide therapy. Hum Pathol 6:693–709, 1975.
12. Kushner JP, Hansen VL, and Hammar SP. Cardiomyopathy after widely-separated courses of adriamycin exacerbated by actinomycin D and mithramycin. Cancer 36:1577–1584, 1975.
13. Minow RA, Benjamin RS, and Gottlieb JA. Adriamycin (NSC-123127) cardiomyopathy—an overview with determination of risk factors. Cancer Chemother Rep Part 3, vol 6(2):195–201, 1975.
14. Weiss AJ, and Manthel RW. Experience with the use of adriamycin in combination with other anticancer agents using a weekly schedule with particular reference to lack of cardiac toxicity. Cancer 40:2046–2052, 1977.
15. Bristow MR, Mason JW, Billingham ME, et al. Doxorubicin cardiomyopathy: evaluation of phonocardiography, endomyocardial biopsy, and cardiac catheterization. Ann Intern Med 88:165–168, 1978.
16. Lefrak EA, Pitha J, Rosenheim S, et al. A clinicopathologic analysis of adriamycin cardiotoxicity. Cancer 32:302–314, 1973.
17. Rinehart JJ, Lewis RP, and Balcerzak SJ. Adriamycin cardiotoxicity in man. Ann Intern Med 81:475–478, 1974.
18. Ramos A, Meyer RA, Korfhagen J, et al. Echocardiographic evaluation of adriamycin cardiotoxicity in children. Cancer Treat Rep 60:1281–1284, 1976.

19. EWY GA, JONES SE, GROVES BM, ET AL. Echocardiographic diagnosis of adriamycin heart disease. Clin Res 23:115A, 1975.
20. MINOW RA, GOTTLIEB J, FRAZIN L, ET AL. Adriamycin cardiotoxicity. Ann Intern Med 82:855–857, 1975.
21. BLUM RH, and CARTER SK. Adriamycin: a new anticancer drug with significant clinical activity. Ann Intern Med 80:249–259, 1974.
22. BENJAMIN RS, WIERNIK PH, and BACHUR NR. Adriamycin chemotherapy—efficacy, safety, and pharmacologic basis of an intermittent single high-dosage schedule. Cancer 33:19–27, 1974.
23. VON HOFF DD, ROZENCWEIG M, LAYARD M, ET AL. Daunomycin-induced cardiotoxicity in children and adults. A review of 110 cases. Am J Med 62:200–208, 1977.
24. VON HOFF DD, LAYARD M, BASA P, ET AL. Analysis of risk factors for development of adriamycin induced congestive heart failure (CHF). Proc Am Assoc Cancer Res and ASCO 19:54, 1978.

Treatment of Cervical Carcinoma With Adriamycin Combined With Methotrexate [1]

Brief Reports and Preliminary Communications

C. Papavasiliou,* J. Pappas, D. Aravantinos, and D. Kaskarelis [2]

At the University of Athens, we have treated several series of patients with carcinoma of the uterine cervix using various chemotherapeutic agents. The results have been generally disappointing whether we used regional (1) or systemic (2) routes of administration or whether combination- or single-agent therapy was used. Combined therapy, however, gave superior results in comparison to single agents (3). Since Guthrie and Way (4) reported improved results using a combination of adriamycin and methotrexate in nine patients, we decided to treat our patients with the same scheme. We report our experience based on 24 patients treated with this regimen.

MATERIALS AND METHODS

A total of 26 patients were treated, but we were only able to evaluate 24. Their ages ranged from 40 to 71 years (mean age, 55). All patients had satisfactory blood examinations and normal liver and renal function tests. All patients had biopsy-proven epidermoid carcinoma and were examined by a team of physicians including a gynecologist, a radiation therapist, and a chemotherapist. Ambulatory patients with stages IIIb and IV were entered in this trial if they had measurable disease. Staging was carried out under general anesthesia according to FIGO recommendations. The patients were evaluated by the same group of physicians 1 month after completion of the second cycle, that is, 2 months after initiation of therapy. After re-evaluation, all but two patients were treated by either irradiation or other chemotherapeutic schemes. Thus, all patients, except for two responders, received only two cycles of chemotherapy. Adriamycin was given at a dose of 50 mg/m² iv on Day 1 and methotrexate was given at a dose of 20 mg/m² on Days 1 and 8. On Day 22, the scheme was repeated. Total regression was defined as the complete disappearance of all lesions while partial regression was defined as a 50% reduction in the size of measurable lesions. Blood examinations were done prior to drug administration and also at re-examination.

RESULTS

The results are shown in table 1. No patient had a complete response. A 50% reduction was noted in seven patients (29%). Factors influencing results were previous therapy and the site of the tumor. Five of ten patients (50%) without previous therapy and two of five patients (40%) with extrapelvic disease had regression. Five responding patients subsequently received irradiation. One patient who developed lung metastases and also probable local recurrence after previous radiation therapy received six cycles with a partial remission that lasted 8 months. Another patient with pulmonary metastases received five cycles and the remission lasted 5 months. Subjective improvement was common. Among 18 patients with vaginal discharge, 17 reported improvement. Among 16 patients with pain, 14 had partial pain relief.

TOXICITY

Drug toxicity was not severe. The observed side effects are listed in table 2. There was one drug-

TABLE 1.—Results of therapy

	No. of patients	No. of responses (%)
Site		
Pelvic	19	5 (26.3)
Extrapelvic	5 (lung [4] and liver [1])	2 (40)
Previous therapy		
With	14 (irradiation [10], irradiation + surgery [3], and irradiation + chemotherapy [1])	2 (14.3)
Without	10	5 (50)
Stage		
IIIb	11	2 (18)
IV	13	5 (38.4)
Totals	24	7 (29.1)

[1] Received Mar 21, 1978; revised June 20, 1978; accepted June 21, 1978.

[2] Departments of Clinical Therapeutics and Obstetrics and Gynecology, Alexandra Hospital, University of Athens, Greece.

Reprint requests to: C. Papavasiliou, MD, Department of Clinical Therapeutics, Alexandra Hospital, University of Athens, 611 Athens, Greece.

TABLE 2.—Toxic effects

Toxic effect	No. of cases
Alopecia	24
Stomatitis	4
Pancytopenia	1
Leukopenia	1
Thrombocytopenia	1
Diarrhea	1

related death. This patient developed pancytopenia and severe hemorrhagic diarrhea. She had been treated previously with other chemotherapeutic agents. There was one case of reversible leukopenia and one case of reversible thrombocytopenia. The mean wbc count before therapy was 7530/mm^3 while the mean nadir wbc count was 4535/mm^3. The platelet counts before and after therapy were 239,000 and 206,000/mm^3 respectively. There was no myocardial toxicity.

Patients tolerated subsequent radiation therapy remarkably well. Three patients received almost full doses of intracavitary radium and external irradiation. Thirteen patients received full doses of external megavoltage irradiation (median tumor dose, 4860 rads in 5–7 weeks by rotational technique). In these patients, the acute side effects of irradiation (diarrhea and cystitis) were not greater than those seen in patients who had not received previous chemotherapy.

DISCUSSION

Epidermoid carcinoma of the uterine cervix is considered to be a chemoresistant lesion since the remission rate is low and the duration of remission is usually short (5). Patients with locally recurrent disease usually have avascular lesions due to previous surgery and/or irradiation. This is another reason for chemotherapeutic failure. Thus, 50% of our patients without previous therapy and 40% of those with extrapelvic disease had remission. The higher remission rate in stage IV patients is partly due to the extrapelvic lesions. Stage IIIb lesions which infiltrate the parametria and are attached or involve the lateral pelvic wall may also be avascular. Evaluating the results of chemotherapy for cervical carcinoma is also difficult. The complex pattern of spread predisposes to the creation of nonmeasurable lesions. In addition, in previously treated patients, fibrotic tissue cannot be easily distinguished from tumors. For this reason, strict criteria for drug evaluation are needed. The present results are inferior to those reported by Guthrie and Way (4). For unknown reasons, the toxicity was also milder.

In the recent literature there is some evidence that schemes including bleomycin may achieve better results than those attained in this study (6,7).

REFERENCES

1. LOUROS N, PAPAVASILIOU C, and DELIYIANNIS E. Palliative treatment of advanced cervical carcinoma by intra-aortic injection of nitrogen mustard. J Obstet Gynecol Br Cwlth 72:610–613, 1965.
2. PAPAVASILIOU CG, ELIAS C, and DESALERMOS P. Methotrexate in advanced cervical cancer. *In* Proceedings of the 5th International Congress of Chemotherapy, 1967, pp 469–475.
3. PAPAVASILIOU C, ANGELAKIS P, GOUVALIS P, ET AL. Treatment of cervical carcinoma by methotrexate (NSC–740) combined with cyclophosphamide (NSC–26271). Cancer Chemother Rep 53:255–261, 1969.
4. GUTHRIE D, and WAY S. Treatment of advanced carcinoma of the cervix with adriamycin and methotrexate combined. Obstet Gynecol 44:586–589, 1974.
5. WASSERMAN T, and CARTER S. The integration of chemotherapy into combined modality treatment of solid tumors. VIII. Cervical cancer. Cancer Treat Rev 4:25–46, 1977.
6. BAKER LH, OPIPARI MI, and IZBICKI RM. Phase II study of mitomycin-C, vincristine, and bleomycin in advanced squamous cell carcinoma of the uterine cervix. Cancer 38:2222–2224, 1976.
7. CONROY JF, LEWIS GC, BRADY LW, ET AL. Low dose bleomycin and methotrexate in cervical cancer. Cancer 37:660–664, 1976.

Treatment of Adult Acute Lymphoblastic Leukemia With Cytosine Arabinoside, Vincristine, and Prednisone [1,2]

Brief Reports and Preliminary Communications

Roger D. Gingrich, James O. Armitage, and C. Patrick Burns [3,4,*]

SUMMARY

Thirteen adults with acute lymphoblastic leukemia were entered into a protocol in which cytosine arabinoside infusion was added to vincristine and prednisone as remission induction and periodic intensification therapy. Central nervous system prophylaxis consisting of cranial irradiation and intrathecal methotrexate was given, and all patients received continuous oral 6-mercaptopurine and methotrexate as maintenance treatment. Myelotoxicity was severe during induction, with prolonged granulocyte and platelet count nadirs noted. Nine of 13 patients (69%) obtained a complete remission and one (8%) obtained a partial remission. The median duration of complete remission was 38.1 weeks. It was concluded that cytosine arabinoside in combination with vincristine and prednisone is an effective but toxic antileukemic regimen which did not produce a major improvement in remission duration.

[Cancer Treat Rep 62:1389–1391, 1978]

Most induction regimens for acute lymphoblastic leukemia (ALL) include vincristine and prednisone which together produce complete remission (CR) in ≥ 90% of children with this disease (1,2). However, these two drugs result in CRs in only about 50%–60% of adults with ALL in most studies (3–5), although two studies have reported a higher success rate (6,7). The addition of daunorubicin, adriamycin, or L-asparaginase results in CRs in 72%–100% of patients (1,3,8–11); however, despite this increased CR rate, duration of the remissions has been disappointingly brief (6,9,10).

Cytosine arabinoside is an effective drug in adult ALL (12). The Southwest Oncology Group, employing a 10-day continuous infusion of cytosine arabinoside in combination with vincristine plus prednisone, obtained a 78% CR rate (13). In an attempt to improve the induction rate and duration of remission, adults with ALL were entered into a study in which cytosine arabinoside was used with vincristine and prednisone for remission induction and for periodic late intensification therapy.

MATERIALS AND METHODS

All previously untreated patients with ALL seen at University of Iowa Hospitals from September 1975 to May 1977 who gave informed consent were enrolled. The diagnosis of ALL was made on the basis of Wright's stain morphology and negative peroxidase stain of the bone marrow. In addition, most patients had PAS-positive blast cells and low serum lysozyme. No patients with undifferentiated or lymphosarcoma cell leukemia were included. The induction scheme included 1.4 mg/m² (not > 2.0 mg) of vincristine iv weekly and 40 mg/m² of prednisone orally daily, each for a total of 4 weeks, and 200 mg/m²/day of cytosine arabinoside on Days 1–5 by continuous iv infusion. If no antileukemic effect was seen in the marrow by Day 14, the cytosine arabinoside infusion was repeated on Days 15–19. Once CR was obtained, 2400 rads of whole brain irradiation and 12 mg/m² of intrathecal methotrexate (not > 20 mg) for five doses spaced over 2 weeks were given as central nervous system prophylaxis to patients < 60 years old. Maintenance therapy was subsequently given: 50 mg/m² of 6-mercaptopurine orally daily and 20 mg/m² of methotrexate orally weekly, with the dose adjusted to keep the wbc count < 4000/mm³. Every 4 months the 6-mercaptopurine and methotrexate were discontinued and the patients received 100 mg/m²/day of cytosine arabinoside by continuous iv infusion on Days 1–5, 1.4 mg/m² of vincristine iv on Day 1, and 40 mg/m² of prednisone

[1]Received Mar 22, 1978; revised July 5, 1978; accepted July 11, 1978.

[2]Supported in part by Public Health Service grant CA-00324 (C. P. Burns) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, and by the Iowa Leukemia and Cancer Research Fund.

[3]Department of Medicine, University of Iowa Hospitals, Iowa City.

[4]We gratefully acknowledge the assistance of house officers, fellows, and attending staff of our Department of Medicine who participated in the care of our patients.

*Reprint requests to: C. Patrick Burns, MD, Department of Medicine, University of Iowa Hospitals, Iowa City, Iowa 52242.

TABLE 1.—Summary of clinical features

Patient No.	Age/ sex	Blast cells/ $mm^3 \times 10^{-3}$	% surface markers: T/B*	2nd course of cytosine arabinoside†	Duration of neutrophil count nadir (days)‡	Complications during induction	Outcome of induction therapy	Duration of remission (wks)	Survival (wks)
1	61/F	0.8	ND§	No	—	Pneumonia, bacteremia with *Klebsiella pnemoniae*	CR	42.1	54.1
2	22/M	0.06	ND	No	21	Bacteremia with *Clostridium putrifacens*	CR	86.4+	89.4+
3	43/M	68.8	2/1	Yes	39	Bacteremia with *Staphylococcus epidermidis*	CR	17.0	58.7
4	46/M	0	ND	Yes	10	Fever with negative cultures	CR	50.0	81.3+
5	21/M	2.2	21/—	Yes	13	Fever with negative cultures	CR	53.6	112.4
6	32/M	0.9	3/4	Yes	37	Pneumonia, bacteremia with *Pseudomonas aeruginosa*	CR¶	1.1	7.4
7	79/M	19.5	19/—	Yes	23	Fever, pneumonia with negative cultures	CR	14.7	14.7
8	60/F	8.0	ND	Yes	6	None	CR**	3.6	6.9
9	31/F	290.0	1.3/4	Yes	5	None	CR	28.7	76.4
10	86/M	4.5	13/4	No	20	None	Partial remission	13.4	17.7
11	63/F	1.1	ND	No	15	Pneumonia with *Candida tropicalis*	Died	—	—
12	20/F	0	ND	Yes	5	Pneumonia, bacteremia with *Escherichia coli;* pulmonary hemorrhage	Died	—	—
13	19/F	3.5	12/2	No	15	Bacteremia with *Pseudomonas aeruginosa*	Died	—	—

*T cells identified by sheep rbc rosettes/B cells identified by surface immunoglobulin.
†Yes = 2nd course of drug was given on Days 15–19; no = not given.
‡< 500 cells/mm^3.
§ND = not done.
¶Died in CR of infection acquired during induction therapy.
**Died in CR of *Klebsiella* sepsis acquired after induction but before maintenance therapy.

orally on Days 1–5. Criteria for evaluating response were those of the Cancer and Leukemia Group B.

RESULTS

There were 13 adults entered in this study with a median age of 43 years. Table 1 details the clinical features and some of the known prognostic factors which were evident in these patients. All patients required platelet transfusions and four received granulocyte transfusions. Nine patients (69%) entered a CR, with a median of 21 days from initiation of therapy to an M_1 marrow. One additional patient (8%) obtained a partial remission. Three patients died during remission induction 12–16 days after beginning therapy without obtaining a CR. They were considered evaluable with respect to the remission rate.

Hematologic toxicity was severe. Six patients developed bacteremias during the granulocyte count nadir ($< 500/mm^3$) which lasted a median of 20 days. Four of these bacteremias were with gram-negative organisms (three patients also had concurrent pneumonia) and, despite appropriate antibiotic therapy, three of these patients died (Nos. 6, 12, and 13). The median duration of the platelet count nadir ($< 20{,}000/mm^3$ without transfusion) was 12 days and it was associated with one death due to pulmonary hemorrhage.

The median duration of the CRs was 38.1 weeks by the life-table analysis method. All relapses were in the bone marrow and no patient had a central nervous system relapse. The median duration of survival for those achieving CR was 56 weeks.

DISCUSSION

In an attempt to improve upon the disappointing experience that has been reported in the treatment of adult ALL, cytosine arabinoside was incorporated into a standard vincristine plus prednisone induction regimen. The design of the trial was based on the successful programs for childhood ALL as follows: (*a*) prednisone was given daily for 28 days; (*b*) vincristine was given weekly for a minimum of five doses; (*c*) patients attaining a CR received central nervous system prophylaxis; and (*d*) continuous oral maintenance therapy was given. In addition, periodic reinforcement with the induction drugs was administered during remission in an attempt to delay previously observed rapid relapses.

The CR rate of 69% is comparable to that reported for other regimens (1,3,10). The remission duration of 38.1 weeks is also comparable to many recent reports (4,6,9). However, it is apparent that the protocol including the periodic re-induction did not result in an extension of the disease-free interval.

Myelotoxicity was severe, resulting in long periods of granulocytopenia. There were febrile episodes in 11 of the 13 patients and seven instances of documented bacteremias. Other investigators have noted unexpectedly high infection rates for regimens containing high-dose steroids (5,6). We suspect that the high rate of life-threatening infections is the result of the immunosuppressive effects of the daily steroids superimposed on the prolonged myelosuppression.

We conclude that the combination of cytosine arabinoside, vincristine, and prednisone is an effective antileukemic regimen. However, the therapy was associated with severe toxicity and it did not lead to improved remission induction rates or extended remissions.

REFERENCES

1. Gee TS, Haghbin M, Dowling MD, et al. Acute lymphoblastic leukemia in adults and children: differences in response with similar therapeutic regimens. Cancer 37:1256–1264, 1976.
2. Simone JV, Aur RJA, Hustu HO, et al. Acute lymphocytic leukemia in children. Cancer 36:770–774, 1975.
3. Lister TA, Whitehouse JMA, Beard MEJ, et al. Combination chemotherapy for acute lymphoblastic leukaemia in adults. Br Med J 1:199–203, 1978.
4. Armitage JO, and Burns CP. Remission maintenance of adult acute lymphoblastic leukemia. Med Pediatr Oncol 3:53–58, 1977.
5. Einhorn LH, Meyer S, Bond WH, et al. Results of therapy in adult acute lymphocytic leukemia. Oncology 32:214–220, 1975.
6. Scavino HF, George JN, and Sears DA. Remission induction in adult lymphocytic leukemia. Use of vincristine and prednisone alone. Cancer 38:672–677, 1976.
7. Curtis JE, Cowan DH, Bergsagel DE, et al. Acute leukemia in adults: assessment of remission induction with combination chemotherapy by clinical and cell-culture criteria. Can Med Assoc J 113:289–294, 1975.
8. Einhorn LH, and Bond WH. Remission induction with daunomycin, vincristine, and prednisone in adult acute lymphocytic leukemia. Oncology 34:25–28, 1977.
9. Shaw MT, and Raab SO. Adriamycin in combination chemotherapy of adult acute lymphoblastic leukemia. Med Pediatr Oncol 3:261–266, 1977.
10. Jacquillat C, Weil M, Gemon MF, et al. Combination therapy in 130 patients with acute lymphoblastic leukemia (protocol 06 LA 66-Paris). Cancer Res 33:3278–3284, 1973.
11. Henderson ES, and Glidewell O. Combination therapy of adult patients with acute lymphocytic leukemia (ALL). Proc Am Assoc Cancer Res and ASCO 15:102, 1974.
12. Bryan JH, Henderson ES, and Leventhal BG. Cytosine arabinoside and 6-thioguanine in refractory acute lymphocytic leukemia. Cancer 33:539–544, 1974.
13. Hewlett JS, Balcerzak S, Gutterman J, et al. Remission induction in adult acute leukemia by 10-day continuous intravenous infusion of ara-C, plus Oncovin and prednisone. Maintenance with and without BCG. Proc Am Assoc Cancer Res and ASCO 16:234, 1975.

Alteration of Binding of the Supravital Dye Hoechst 33342 to Human Leukemic Cells by Adriamycin [1,2]

Brief Reports and Preliminary Communications

Harvey D. Preisler [3,4,*]

Flow cytofluorometry is a method which permits the rapid analysis of cells tagged with a fluorescent probe. We have been using this method to study the interaction of a supravital dye (Hoechst 33342 [H33342]) with human hematopoietic cells. This dye has been reported by Arndt-Jovin and Jovin (1) to reversibly bind to the DNA of Friend erythroleukemia cells. In our studies of patients with acute leukemia being treated with adriamycin, we observed that the latter chemotherapeutic agent interferes with the interaction of H33342 with leukemic cells. Further studies demonstrated a linear relationship between the effects of adriamycin on the uptake and/or binding of H33342 and, hence, suggested that the interference of the interaction of H33342 with cells induced by adriamycin may provide a sensitive means for studying the interaction of this chemotherapeutic agent with human cells.

MATERIALS AND METHODS

Human bone marrow cells, both normal and leukemic, were obtained as previously reported (2). The cells were suspended in RPMI-1640 made 10% with fetal calf serum (complete media) at a concentration of 5×10^6 or 10^7 cells/ml and incubated for 1 hour in a humidified incubator made 5% with CO_2 at 37°C. After 1 hour, adriamycin in complete media was added to make the appropriate final concentrations in the cell suspension. Incubation was continued for the appropriate period of time: the cells were then washed once and incubated for 2 hours with H33342 (20 μmols/ml) in complete media. The cells were pelleted, resuspended at 2×10^6 cells/ml in sterile, filtered phosphate-buffered saline, and analyzed by a Becton-Dickinson FACS-II using a wavelength of 351 nm.

RESULTS

Patient 1 is a patient with acute myelocytic leukemia who was treated with the combination of adriamycin and cytosine arabinoside for remission induction therapy (3). Figure 1 gives the DNA histogram of the patient's bone marrow prior to treatment and 24 hours after therapy was begun. Note that while the configuration of the histogram is unchanged, the channel at which the G_1 peak is located in the marrow obtained 24 hours after the initiation of treatment is less than that for the pretreatment marrow.

Human leukemic bone marrow cells were incubated with different concentrations of adriamycin for different lengths of time in vitro and then incubated with H33342. Figure 2a illustrates that there is an inverse exponential relationship between the amount of adriamycin to which the cells were exposed and the G_1 peak channel number. This relationship holds true for as little as 0.25 μg of adriamycin/ml. A similar relationship exists for various durations of cell exposure to adriamycin for up to 120 minutes (fig 2b). Incubation with up to 10 μg/ml of cytosine arabinoside had no effect on the G_1 peak channel number nor did the presence of this drug influence the effect of adriamycin.

DISCUSSION

H33342 is a benzimidazole dye which reversibly binds to DNA (1). Its uptake is time-, concentration-, and, to some extent, temperature-dependent (1).[5] Adriamycin interferes with the uptake and/or the binding of this dye to DNA. This effect appears to be stoichiometric for cells incubated with as little as 0.25 μg/ml of adriamycin for 1 hour, thus permitting interference with H33342 binding to be used as a means of quantitating the amount of adriamycin present.

[1]Received Apr 13, 1978; revised June 9, 1978; accepted June 19, 1978.

[2]Supported by Public Health Service grants CA-05834 and CA-13038 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Department of Medicine A, Roswell Park Memorial Institute, Buffalo, NY.

[4]I would like to thank Ms. Irene Walczak and Mr. George Hausner for their technical assistance and Dr. Loewe of the Hoechst Co for supplying the H33342.

*Reprint requests to: Harvey D. Preisler, MD, Department of Medicine A, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

[5] Preisler HD. Manuscript in preparation, 1978.

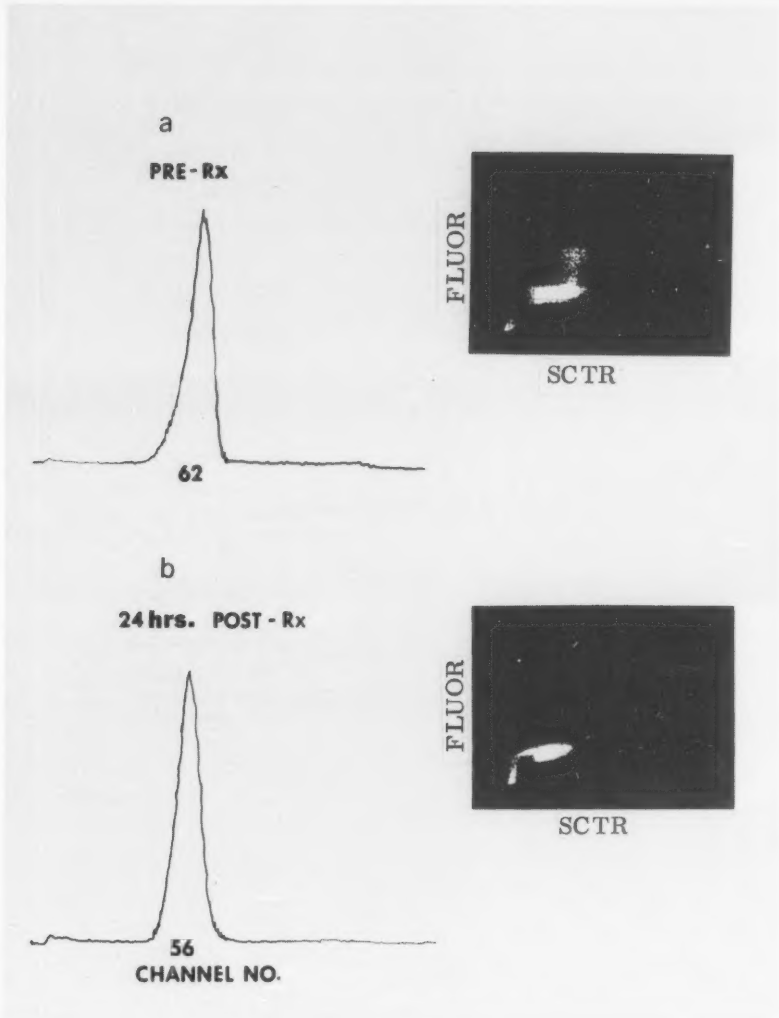

FIGURE 1.—(*a*) = pretherapy DNA histogram and light scatter-DNA profile of the leukemic marrow cells of patient 1; (*b*) = same data 24 hrs after therapy was begun. The ordinate of the DNA histogram represents cell number and the abscissa represents fluorescence intensity. Note that the G_1 peak has shifted downward from channel 62 to 56. Also note the disappearance of a cell population in the light scatter-DNA plot. These cells were large with a >2N content of DNA.

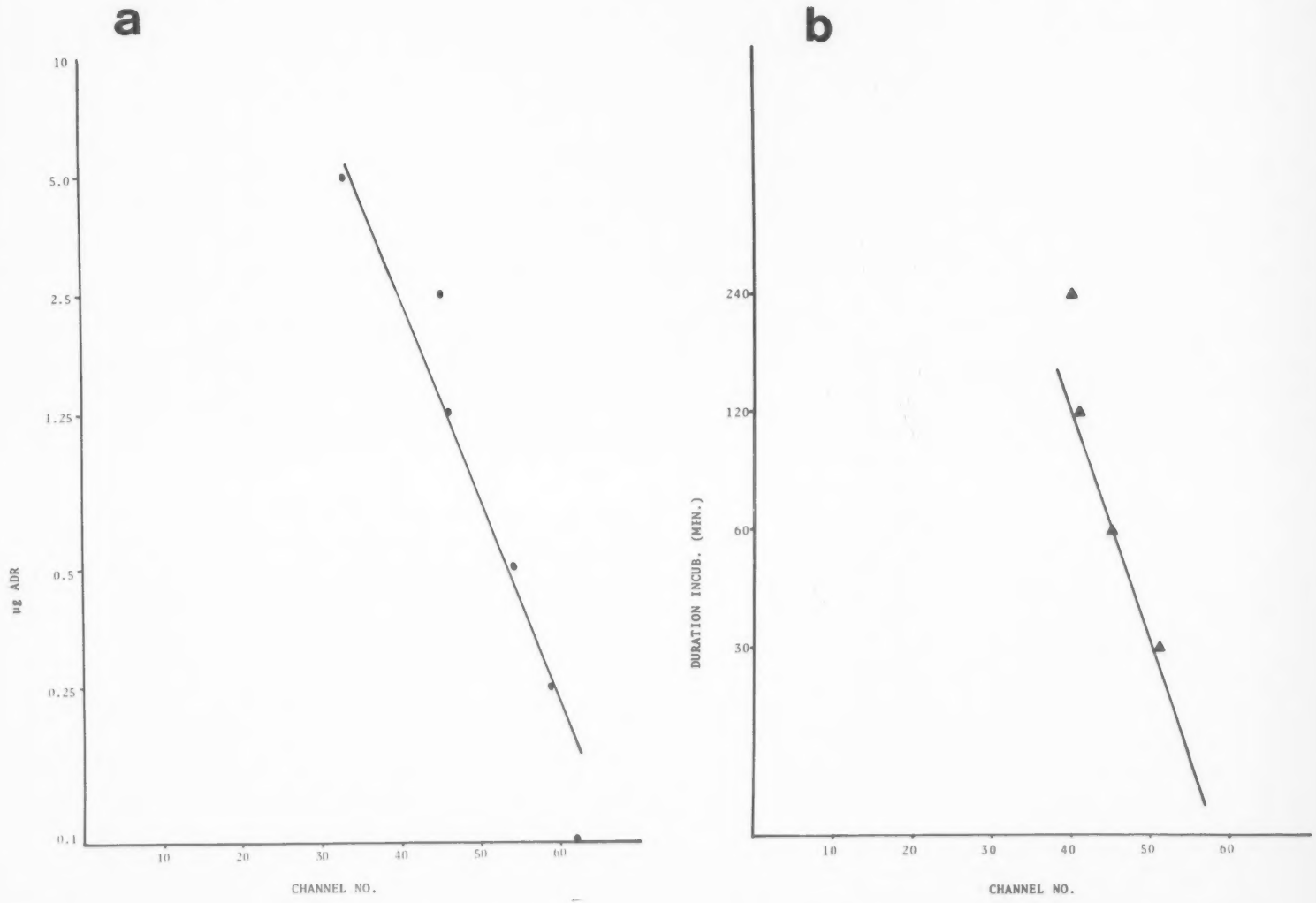

FIGURE 2.—(*a*) = relationship between channel number of G_1 peak and concentration of adriamycin to which the cells were exposed prior to incubation of cells with H33342; (*b*) = relationship between channel number of G_1 peak and duration of incubating cells with 2.5 µg/ml of adriamycin prior to incubation of cells with H33342.

Within 24 hours after receiving a single dose of adriamycin, the G_1 fluorescence of marrow cells of four of seven patients was less than it was prior to initiation of therapy. These data, together with those described above, suggest that this interference phenomenon may provide a simple means of studying the interaction in vivo of adriamycin and human cells during chemotherapy. The mechanism of this interference phenomenon is not known but could involve competition for binding sites on DNA, adriamycin-induced metabolic interference of the uptake of H33342, or an energy exchange between adriamycin and H33342 if both are simultaneously bound to cellular DNA. One additional potential application for the observations reported here would be a biologic assay to quantitate the amount of adriamycin present in serum or other body fluids. This could be effected by incubating an adriamycin-sensitive cell line with the body fluid in question, followed by incubation with H33342 and measurement of the effects of such incubation of G_1 fluorescence.

REFERENCES

1. ARNDT-JOVIN DJ, and JOVIN TM. Analysis and sorting of living cells according to DNA content. J Histochem Cytochem 25:585–589, 1977.
2. PREISLER HD, WALCZAK I, RENICK J, ET AL. Separation of leukemic cells into proliferative and quiescent subpopulations by centrifugal elutriation. Cancer Res 37:3876–3880, 1977.
3. PREISLER HD, BJORNSSON S, and HENDERSON ES. Adriamycin-cytosine arabinoside therapy for adult acute myelocytic leukemia. Cancer Treat Rep 61:89–92, 1977.

Invasive Thymoma Responsive to Oral Corticosteroids [1]

Judd Shellito, Janardan D. Khandekar,* William P. McKeever, and Nicholas A. Vick [2]

Traditionally, thymomas are treated by surgery and/or radiation. The therapy for recurrent thymomas has not been systematically studied. In this paper we report on a patient with recurrent invasive thymoma who had a complete remission after treatment with oral corticosteroids.

CASE REPORT

In November 1974, an anterior mediastinal tumor was discovered on a routine chest roentgenogram given to a 52-year-old white man. A 70-g well-encapsulated mixed lymphoepithelial thymoma was resected from the anterior mediastinum in January 1975. There was no invasion of surrounding structures by the tumor. At the time of surgery, the patient was free of neuromuscular symptoms.

In February 1975, the patient had symptoms and signs of myasthenia gravis. The diagnosis was confirmed by a positive edrophonium (Tensilon) test. He responded well to neostigmine for the next 10 months.

In December 1975, the patient was admitted to the hospital because of acute sigmoid diverticulitis. His chest roentgenogram at the time of admission showed a new anterior mediastinal tumor with extension laterally to both hila. Transthoracic needle biopsy of the tumor revealed a patchy lymphocytic infiltrate compatible with recurrent thymoma. He was treated with mediastinal radiation of 5250 rads with complete regression of the tumor.

On March 11, 1977, a 3-cm pleural tumor was discovered in the left mid-lung field on routine chest roentgenogram (fig 1). This was felt to be recurrent thymoma. The patient refused a repeat transthoracic needle biopsy and then began treatment with prednisone at a dose of 60 mg/day. On March 30, 1977, a followup chest roentgenogram showed the pleural tumor to be reduced in size. Because of cushingoid side effects, the patient was at that time switched to prednisone at a dose of 40 mg every other day. Chest roentgenograms given on May 3, 1977, demonstrated that the pleural tumor had returned to its former size. The patient was then given prednisone at a dose of 40 mg/day. Subsequent chest roentgenograms showed a gradual shrinkage of the tumor, until its complete disappearance was noted on a chest roentgenogram given on June 24, 1977 (fig 2). At the same time, the patient reported worsening symptoms of myasthenia gravis and anti-acetylcholine receptor protein antibody levels were elevated at 120×10^{-9} M (normal = up to 0.6×10^{-9} M, courtesy of Children's Hospital of San Francisco, Calif).

In November 1977, the patient was admitted to the hospital for the evaluation of epigastric pain, a 5-pound weight loss, and progressive unexplained pedal edema. The prednisone dose (40 mg/day) had remained stable since May 1977. A 15-cm hard tumor was palpable in the epigastrium. Pitting edema was present to mid-calf. There was no jugular venous distention. An exploratory laparotomy was performed, and the tumor was found to be thymoma metastatic to the liver with compression of the inferior vena cava. Two days after surgery, the patient suffered a cardiac arrest and died.

An autopsy showed malignant thymoma in the mediastinum with metastases to the liver, both lungs, inferior vena cava, and posterior myocardium. Tumor was found as a pleural implant at the site of roentgenographic abnormality. There was no histologic evidence of tumor necrosis within the pleural implant.

DISCUSSION

Thymic malignancy cannot be established on the basis of histology alone (1). Most investigators agree that encapsulation is the best histologic criterion of a benign tumor (1–3). However, even well-encapsulated tumors may become invasive, as demonstrated in our patient. In addition to encapsulation, thymomas may be classified as benign if there is no tissue invasion at the time of surgical exploration. Using

[1]Received Mar 1, 1978; revised June 19, 1978; accepted July 5, 1978.

[2]Departments of Medicine and Neurology, Northwestern University School of Medicine, and Divisions of Cardiology, Neurology, and Oncology, Evanston Hospital, Ill.

**Reprint requests to:* Janardan D. Khandekar, MD, Division of Oncology, Evanston Hospital, 2650 Ridge Ave, Evanston, Ill 60201.

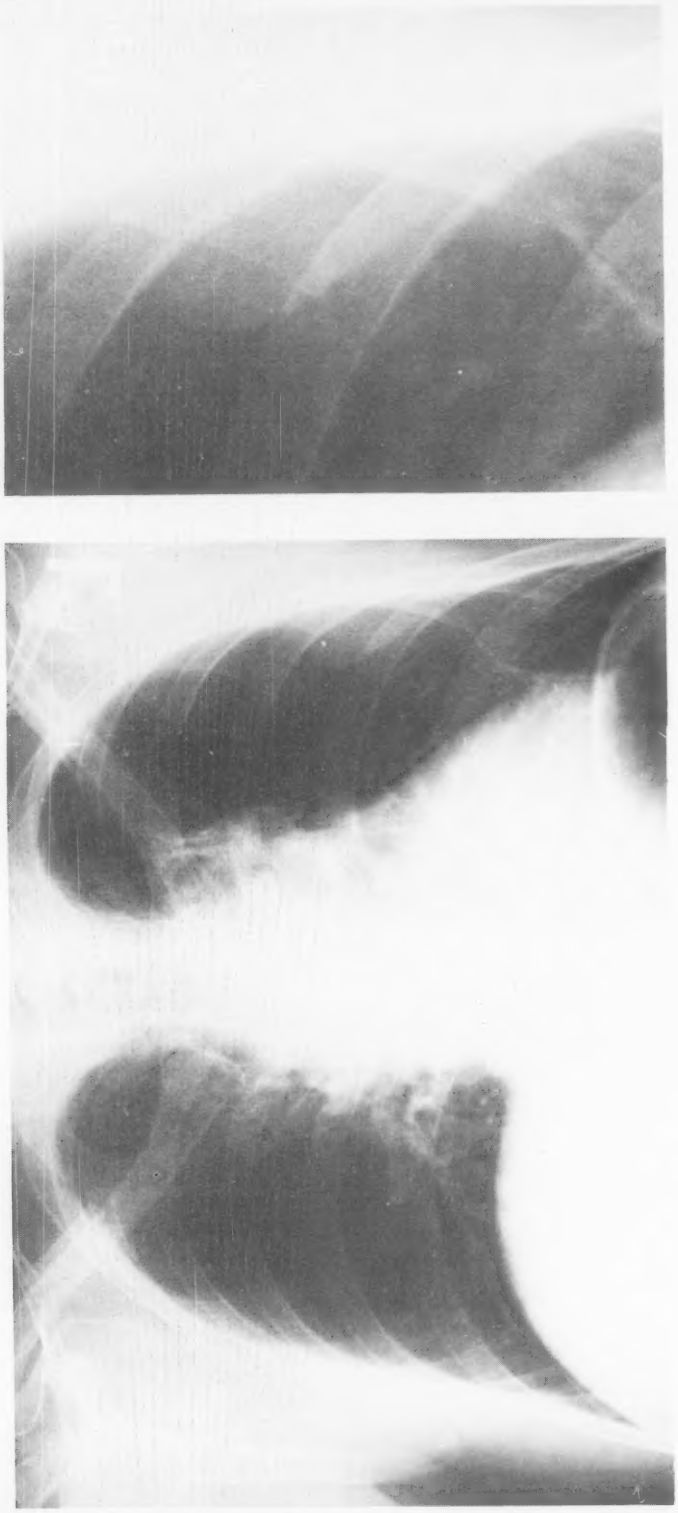

FIGURE 1.—Chest roentgenogram given on Mar 11, 1977, showing left-sided pleural tumor (arrow).

FIGURE 2.—Chest roentgenogram given on June 24, 1977, after 3 mos of prednisone therapy, showing disappearance of pleural tumor.

these criteria, the majority of thymomas are malignant at the time of presentation (3). There is a well-recognized association between thymoma and myasthenia gravis (as in our patient), red-cell hypoplasia, Addison's disease, Cushing's syndrome, acquired hypogammaglobulinemia, rheumatoid arthritis, systemic lupus erythematosus, and other malignancies. The presence of one or more of these associated syndromes worsens the prognosis (2).

The initial therapy for a thymoma is surgical excision (4). If the tumor cannot be surgically removed, is nonencapsulated, or is only partially encapsulated, a course of radiation therapy to the mediastinum should be administered (3,4). If the tumor recurs after surgical removal, most authorities advise radiotherapy, although Fechner (5) argues that re-exploration may allow a second surgical excision. Thymomas recur most commonly in the mediastinum. The next most common location of recurrent tumor is a pleural implant, as shown in our patient (5). Extrathoracic metastases, such as those seen in our patient, are distinctly uncommon (6,7).

Chemotherapy for invasive thymoma has not been systematically evaluated. Both single-agent and combination chemotherapeutic regimens have been used. Short-term remissions have been achieved with single-agent use of cyclophosphamide, *cis*-dichlorodiammineplatinum(II), and adriamycin (4,8). Combination chemotherapy of the MOPP type (mechlorethamine, vincristine, prednisone, and procarbazine) with and without the addition of bleomycin, adriamycin, or CCNU has been proposed (8,9), but its exact role remains to be defined.

Corticosteroids are often used in patients with thymomas for the associated symptoms of myasthenia gravis rather than as a form of treatment for the tumor. Green and Forman (10) described an instance of tumor regression after the use of corticosteroids, but this was a benign thymoma and was confined to the mediastinum. In 1975, Haussen et al (11) suggested that corticosteroids might be effective in the treatment of thymoma based on the treatment of six patients with intractable myasthenia gravis and malignant thymoma. Recently, Posner et al (12) demonstrated an oncolytic effect of

corticosteroids in a case of malignant thymoma metastatic to the epidural space.

It is of interest that glucocorticoid receptors are present in radiation-induced mouse thymoma (13). Although the presence of these receptors in human thymomas has not been demonstrated, it is conceivable that the oncolytic effect of corticosteroids may be mediated through the corticosteroid receptors. In our patient, the regrowth of the tumor seen with a change from daily to alternate-day corticosteroid therapy may indicate that the oncolytic effect of corticosteroids requires constant binding to the corticosteroid receptor. The eventual tumor dissemination seen in our patient while he was still receiving corticosteroid therapy could represent resistance to corticosteroids. It is conceivable that corticosteroids initially caused lysis of the steroid-sensitive thymoma cells, later followed by proliferation of a steroid-resistant neoplastic clone. Corticosteroids can act to select out steroid-resistant tumor cells, as has been demonstrated in murine lymphoma cells (14).

Corticosteroids may be of particular benefit in the rapid lysis of tumors causing compression of the spinal cord or superior vena cava. The high doses of corticosteroids necessary along with a daily dosage schedule limit the use of corticosteroids as initial therapy for recurrent thymoma, but these agents may be of value as palliative therapy alone or in conjunction with radiotherapy.

REFERENCES

1. WEISSBERG D, GOLDBERG M, and PEARSON FG. Thymoma. Ann Thorac Surg 16:141–147, 1973.
2. SALYER WR, and EGGLESTON JC. Thymoma: a clinical and pathological study of 65 cases. Cancer 37:229–249, 1976.
3. BATATA MA, MARTINI N, HAVOS A, ET AL. Thymomas: clinicopathologic features, therapy, and prognosis. Cancer 34:389–396, 1974.
4. GOLDMAN AJ, HERRMANN C, JR, KEESEY J, ET AL. Myasthenia gravis and invasive thymoma: a 20-year experience. Neurology 25:1021–1025, 1975.
5. FECHNER RE. Recurrence of noninvasive thymomas. Cancer 23:1423–1427, 1969.
6. GUILLAN RA, ZELMAN S, SMALLEY R, ET AL. Malignant thymoma associated with myasthenia gravis, and evidence of extrathoracic metastases. Cancer 27:823–830, 1971.
7. SANDERS DB, IGNATIADIS PD, and SWEET DE. Thymoma with distant metastases. Trans Am Neurol Assoc 101:286–288, 1976.
8. BOSTON B. Chemotherapy of invasive thymoma. Cancer 38:49–52, 1976.
9. PONS A, ARMAND JP, VOIGT J, ET AL. Radiotherapy results in 14 cases of malignant thymomas. Bull Cancer (Paris) 64:79–92, 1977.
10. GREEN JD, and FORMAN WH. Response of thymoma to steroids. Chest 65:114–116, 1974.
11. HAUSSEN S, NIBBELINK D, HACKETT E, ET AL. Effect of adrenocorticosteroid therapy of myasthenia gravis on associated malignant thymomas. (Abstr) Neurology 25:347, 1975.
12. POSNER J, HOWIESON J, and CVITKOVIC E. "Disappearing" spinal cord compression: oncolytic effect of glucocorticoids (and other chemotherapeutic agents) on epidural metastases. Ann Neurol 2:409–413, 1977.
13. LEINEN JG, WITTLIFF JL, KOSTYU JA, ET AL. Glucocorticoid-binding components in an irradiation-induced thymoma of the C57BL/6J mouse. Cancer Res 34:2779–2783, 1974.
14. SIBLEY CH, and TOMKINS GM. Mechanisms of steroid resistance. Cell 2:221–227, 1974.

Phase II Study of Melphalan in Colorectal Carcinoma [1]

Joseph DiBenedetto, Jr,[2,3,*] Houshang Moayeri,[3] James T. Evans,[3] and Arnold Mittelman[3,4]

SUMMARY

In a prospective phase II study, 25 patients with advanced (Duke's D) colorectal adenocarcinoma received 0.25 mg/kg of melphalan orally daily for 4 days every 28 days. There were 17 men and eight women. All patients had measurable areas of known malignant disease which served as objective indicators of the response to chemotherapy. All patients were evaluated for at least two cycles of therapy. Each patient had had previous treatment with 5-fluorouracil and, in addition, 24 of 25 patients had had treatment with methyl-CCNU; all patients had disease progression with both regimens. Toxicity consisted of mild gastrointestinal symptoms and transient leukopenia (wbc count $< 4000/mm^3$) in nine of 25 (36%) patients and thrombocytopenia (platelet count $< 100,000/mm^3$) in six of 25 (24%). One of 25 (4%) patients had an objective response for 12 weeks, 15 of 25 (60%) had disease progression, and nine of 25 (36%) remained stable for 2 months. We conclude that melphalan is ineffective for patients with metastatic colorectal carcinoma who have previously failed to respond to both 5-fluorouracil and methyl-CCNU.

[Cancer Treat Rep 62:1401–1402, 1978]

Large bowel carcinoma (colon and rectum) afflicts more patients than any other malignant disease excluding skin cancer, and chemotherapy for this malignancy has not been overwhelmingly successful. 5-Fluorouracil (5-FU) is the most extensively studied drug and has a response rate for metastatic adenocarcinoma of only 21% and a median response duration of only 20 weeks (1,2). Those patients who have failed with 5-FU have generally had less favorable results with other available agents (3).

Melphalan (L–PAM) is an alkylating agent that has been successfully employed in therapy for myeloma. It has recently been reported to have some degree of effectiveness against solid tumors, specifically breast, ovary, and colon (4–7). To fully test the effectiveness of L–PAM against metastatic colorectal carcinoma, we investigated the drug in a prospective phase II study in patients who had failed to respond to previous therapy.

MATERIALS AND METHODS

Patient Selection

Twenty-five patients with histologically confirmed metastatic adenocarcinoma (Duke's D) of the colon or rectum were treated with L–PAM. All patients were ambulatory outpatients who were maintaining reasonable states of nutrition and hydration prior to the study. The age, sex, site of primary tumor, and prior therapy of these patients are shown in table 1. All patients had had disease progression with the previous regimens. None had received either radiation or chemotherapy within 1 month of entry in this study. Chemotherapy was deferred for 4 weeks after an abdominal operation. Each patient had a measurable malignant lesion which was used as an indicator of objective response to treatment. Discrete lesions on chest roentgenogram or palpable masses clearly measurable with a ruler were considered acceptable. All patients were evaluated for at least two cycles of therapy.

Drug Administration

L–PAM was given orally in the dosage of 0.25 mg/kg/day × 4 days and repeated every 28 days. Following treatment, leukocyte and platelet counts and hematocrit values were obtained weekly. Patients were evaluated at 4-week intervals and re-treated until there was objective evidence of progression of malignant disease. The dose was reduced if excessive toxicity occurred following the initial course.

[1]Received Mar 17, 1978; revised June 19, 1978; accepted June 29, 1978.

[2]American Cancer Society Fellow.

[3]Department of Surgical Developmental Oncology, Roswell Park Memorial Institute, Buffalo, NY.

[4]We wish to thank Pat Bourke, RN, and Pat Stronka for their assistance.

*Reprint requests to: Houshang Moayeri, MD, Department of Surgical Developmental Oncology, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

TABLE 1.—Characteristics of patients studied

No. of patients	
Men	17
Women	8
Age in yrs	
Median	64
Mean	61.5
Range	22–77
No. of patients with site of primary tumor in—	
Colon	17
Rectum	8
No. of patients with previous treatment (drugs used alone or in combination) with—	
5-FU	25
Methyl-CCNU	24
β-2'-Deoxythioguanosine	5
DTIC	5
Vincristine	5
Radiation	2
Other phase II drugs	6

Assessment of Response

Objective response was defined as a reduction of ≥ 50% of the product of the two longest perpendicular diameters of the most clearly measurable tumor. If the liver was the primary indicator lesion, a reduction of ≥ 30% of the sum of the measurements below each costal margin constituted an objective response. Progressive disease was defined as an increase of ≥ 25% in any measurable lesion, or new areas of malignant disease, or significant deterioration in symptomatology or performance status.

RESULTS

Toxicity

Toxicity consisted of mild gastrointestinal symptoms; however, all patients were able to tolerate two cycles of the drug. The hematologic toxic effects consisted of leukopenia (wbc count < 4000/mm^3 in 9 patients and < 3000/mm^3 in three patients) and thrombocytopenia (platelet count < 150,000/mm^3 in 12 patients and < 100,000/mm^3 in six patients). There was no evidence of nephrotoxic or hepatotoxic effects.

Therapeutic Response

Only one of 25 (4%) patients had an objective response of 12 weeks' duration. However, nine of 25 (36%) patients had remained stable for 2 months before disease progression was noted. Fifteen of 25 (60%) patients had disease progression within the initial two cycles of L–PAM.

DISCUSSION

Experience with L–PAM in colorectal carcinoma is quite limited. Bullen et al (7) reported a response in two of 13 patients with L–PAM used as initial treatment for metastatic colorectal carcinoma. Carter and Friedman (6), however, reported a response in seven of 28 (25%) patients with colorectal carcinoma. This response rate, if reproducible, would be comparable to the best results obtained with 5–FU (1). In this study, the response rate in a population of patients previously treated with chemotherapy and radiation was only 4% (one of 25 patients). The drug's toxicity, however, was mild and its ease of administration was well accepted. Although the response rate was low, it should be noted that all patients had failed with prior 5–FU therapy. In addition, 24 of 25 patients had prior therapy with methyl-CCNU, a drug believed to act at least in part by alkylation. It has been clearly demonstrated that the previously treated patient is less likely to respond than the patient offered per primum treatment (3). Since chemotherapy for colorectal carcinoma has not been highly successful, and the best single agent, 5–FU, has a response rate of only 21%, further trials of other drugs such as L–PAM used in single-agent or combination therapy in previously untreated patients are desirable. However, from the present study it seems that L–PAM is ineffective in patients previously treated with both 5–FU and methyl-CCNU.

REFERENCES

1. CARTER SK. Large bowel cancer—the current status of treatment. J Natl Cancer Inst 56:3–10, 1976.
2. HAHN RG, MOERTEL CG, SCHUTT AJ, ET AL. A double blind comparison of intensive course 5–FU by oral vs. intravenous route in the treatment of colorectal carcinoma. Cancer 35:1031–1035, 1975.
3. MOERTEL CG. Clinical management of advanced gastrointestinal cancer. Cancer 36:675–682, 1975.
4. FISHER B, CARBONE P, ECONOMOU SG, ET AL. L-Phenylalanine mustard (L–PAM) in the management of primary breast cancer. A report of early findings. N Engl J Med 292:117–122, 1975.
5. DEVITA VT, WASSERMAN TH, YOUNG RC, ET AL. Perspectives on research in gynecologic oncology: treatment protocols. Cancer 38:509–525, 1976.
6. CARTER SK, and FRIEDMAN M. Integration of chemotherapy into combined modality treatment of solid tumors. II. Large bowel carcinoma. Cancer Treat Rev 1:111–129, 1974.
7. BULLEN BR, GILES GR, MALHOTRA A, ET AL. Randomized comparison of melphalan and 5-fluorouracil in the treatment of advanced gastrointestinal cancer. Cancer Treat Rep 60:1267–1271, 1976.

LETTERS

Evaluation of a Chemotherapeutic Regimen for Primary Liver Cancer in Children[1]

Eighteen children with primary liver cancer were admitted to St. Jude Children's Research Hospital (Memphis, Tenn) between 1962 and 1976. Eleven children had hepatoblastoma; four of the five long-term survivors had their tumor completely resected. Seven children had hepatocellular carcinoma; one 10-year survivor was also treated with complete surgical resection. The consistently poor prognosis for those patients with unresectable primary liver cancer (1) led us to develop a chemotherapeutic regimen with the hope of enhancing the effectiveness of combined modality therapy for advanced disease.

Five white boys, 10 months to 16 years old, with unresectable disease involving both lobes of the liver were entered in the study between August 1975 and October 1976 (table 1). Pulmonary metastases were present in two of these boys and peritoneal implants were documented in a third child. One patient had multiple bony metastases. None of these children had had prior radiotherapy or chemotherapy. After obtaining informed consent, 1 mg/m^2 of vincristine was given iv on Day 1, followed by 20 mg/m^2 of adriamycin iv and 100 mg/m^2 of BCNU iv on Day 2 and 400 mg/m^2 of 5-fluorouracil (5-FU) iv on Day 3. Vincristine and 5-FU were repeated on Days 8 and 15, and the entire cycle was repeated on Day 29.

A partial response (> 50% reduction of tumor size) of 3 months' duration was obtained in one child with hepatoblastoma. Stable disease lasting 4, 7, and 12 months without progression was seen in three patients. One patient did not respond.

The chemotherapy for our patients involved the administration of four agents, each with different mechanisms of action and with demonstrated effectiveness against liver cancer (2–5). Although the agents were scheduled so that additive toxicity would be minimized, our first patient developed severe drug-related complications which warranted repeated hospitalizations and successive dose reductions. Increased toxic reactions in this patient may have been age-related. A reduction of drug doses may be warranted for infants until the pharmacokinetics of anticancer agents in young children are known. The severe toxic effects experienced by the first patient might also be attributed in part to the effects of chemotherapy on the regenerating liver, since treatment was started 2 weeks after a right hepatic lobectomy, well within the 8–10-week period of expected rapid hypertrophy following resection. In subsequent patients, toxic effects were limited to cyclic episodes of anemia, and, in one instance, thrombocytopenia (patient 3).

In the present study, a transient partial response was obtained in one of five pediatric patients with primary liver cancer treated with one of the possible

[1]Supported by Public Health Service grants CA-21765 and CA-08151 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, and by ALSAC.

TABLE 1.—Patient characteristics

Patient No.	Age (yrs/mos)	Diagnosis	Metastases	αFP/CEA*	No. of chemotherapy courses	Response	Survival† (mos)
1	1/0	Hepatoblastoma	Pulmonary	Negative/4.9	3	Partial	4
2	0/10	Hepatoblastoma	None	Positive/4.25	4	Stable → progressive disease	11
3	16/0	Hepatocellular carcinoma	Peritoneum	Negative/3.9	12	Stable → progressive disease	23
4	10/3	Hepatocellular carcinoma	Pulmonary, bony, and epidural	Negative/1.8	1	Progressive disease	10
5	11/9	Hepatocellular carcinoma	None	Positive/1.1	7	Stable → progressive disease	15

*αFP = alpha-fetoprotein and CEA = carcinoembryonic antigen.
†From diagnosis.

permutations of these four agents. That response occurred in the patient for whom the drug doses were intolerably high. The incorporation of higher doses of adriamycin (4) into the combination should be considered along with the formulation of different dose schedules in an attempt to increase the therapeutic index.

REFERENCES

1. EXELBY PR, FILLER RM, and GROSFELD JL. Liver tumors in children in the particular reference to hepatoblastoma and hepatocellular carcinoma. American Academy of Pediatrics Surgical Section Survey—1974. J Pediatr Surg 10:329–337, 1975.
2. LASCARI AD. Vincristine therapy in an infant with probable hepatoblastoma. Pediatrics 45:109–112, 1970.
3. MOERTEL CG, SCHUTT AJ, REITEMEIER RJ, ET AL. Therapy for gastrointestinal cancer with the nitrosoureas alone and in drug combination. Cancer Treat Rep 60:729–732, 1976.
4. OLWENY CLM, TOYA T, KATONGOLE-MBIDDE E, ET AL. Treatment of hepatocellular carcinoma with adriamycin. Preliminary communication. Cancer 36:1250–1257, 1975.
5. MOERTEL CG. The liver. *In* Cancer Medicine (Holland JF, and Frei E III, eds). Philadelphia, Lea and Febiger, 1974, pp 1541–1546.

Neil N. Senzer
William Terrell
Charles B. Pratt

St. Jude Children's Research Hospital
PO Box 318
332 N Lauderdale
Memphis, Tenn 38101

July 5, 1978

Surgically Proven Pulmonary Metastases Not Demonstrated by Computed Chest Tomography

The excision of pulmonary metastases from selected patients with sarcomas may result in prolonged survival (1). The selection of patients for thoracotomy has been refined by computed chest tomography (CT), a more precise diagnostic assessment than conventional chest roentgenograms (CXR) or whole-lung tomograms (WLT) (2). However, we report here five patients in whom CT underestimated the extent of biopsy-proven metastatic involvement.

CASE REPORTS

Patient 1 is a 16-year-old male with osteogenic sarcoma (OGS) in the left femur and a normal CXR. However, CT showed four nodules in the left lung and three nodules in the right lung. The lesions measured 5–15 mm. At thoracotomy, there were diffuse deposits of OGS (1–15 mm) throughout the left lung.

Patient 2 is an 18-year-old female with OGS in the skull. Four years after diagnosis, a CXR showed a left lung nodule. CT identified the lesion and two additional right lung nodules. At surgery, multiple deposits of OGS (5–25 mm) were observed in the right lung, parietal pleura, and pericardium.

Patient 3 is a 65-year-old male with a destructive lesion of the proximal femur roentgenographically consistent with OGS. The CXR showed bilateral nodules. CT identified six lesions in the right lung and seven lesions in the left lung. However, at thoracotomy there were literally thousands of 2–3-mm nodules of OGS in the left lung.

Patient 4 is a 24-year-old male with a giant cell tumor of the sacrum. Three years after diagnosis, a CXR revealed two nodules in the right lung. CT demonstrated five additional lesions in the right lung and one nodule in the left lung. At thoracotomy, 30–40 nodules ranging in size from 1 to 6 mm were noted in the right lung.

Patient 5 is a 20-year-old female with neurofibrosarcoma of the thigh. Thirteen months after diagnosis, a CXR indicated a left lung lesion; CT identified single bilateral pulmonary nodules. At thoracotomy, six nodules (3–5 mm) were removed from the right lung and two nodules from the left lung (3 mm and 10 mm, respectively).

DISCUSSION

Conventional CXRs have been the traditional modality for the detection of pulmonary metastases. In patients with normal CXRs, WLT has detected pulmonary lesions in approximately 18% of patients with diverse neoplasms (3). CT has added an innovative diagnostic dimension and detected lung nodules in 11 of 23 patients which were not identified by CXR or WLT (4). This observation is especially important since adjuvant chemotherapy following thoracotomy for OGS is of reported benefit for selected patients (5). However, despite the accuracy of CT, there have been instances where more pulmonary metastases were found at thoracotomy than were demonstrated with CT. In one series of 52 patients, two lesions were missed because of faulty positioning of the patients, but two additional nodules were simply not detected and one of these measured 4 mm (2). This observation and our findings suggest that CT may be relatively insensitive for detecting lesions measuring < 5 mm, although calcified nodules only 2–3 mm in diameter have been imaged by CT (6). There are two major reasons for this insensitivity. The first is the "partial volume" effect. The CT number for a picture element

(pixel) is determined by measuring the attenuation of X-rays by the tissue in a 13-mm thick section of lung. The attenuation produced by an uncalcified nodule < 5 mm in diameter is not great enough to alter the CT number in the corresponding pixels of the CT image and therefore the mass will not be visualized as a nodule. The second important reason is variation in the level of the patient's respiration from one scan to the next. Since the scan thickness is only 13 mm, some nodules may never be included in a scan. That is, due to different levels of inspiration during consecutive scans, a nodule may be above the level being scanned during one scan and then below the level being scanned during the next scan or vice versa.

REFERENCES

1. MARTINI N, HUVOS AG, MIKÉ V, ET AL. Multiple pulmonary resections in the treatment of osteogenic sarcoma. Ann Thorac Surg 12:271–280, 1971.
2. MUHM JR, BROWN LR, and CROWE JK. Use of computed tomography in the detection of pulmonary nodules. Mayo Clin Proc 52:345–348, 1977.
3. NEIFELD JP, MICHAELIS LL, and DOPPMAN JL. Suspected pulmonary metastases. Correlation of chest x-ray, whole lung tomograms, and operative findings. Cancer 39:383–387, 1977.
4. MUHM JR, BROWN LR, and CROWE JK. Detection of pulmonary nodules by computed tomography. AJR 128:267–270, 1977.
5. ROSEN G, HUVOS AG, MOSENDE C, ET AL. Chemotherapy and thoracotomy for metastatic osteogenic sarcoma. Model for adjuvant chemotherapy and rationale for timing of thoracic surgery. Cancer 41:841–849, 1978.
6. KOLLINS SA. Computed tomography of the pulmonary parenchyma and chest wall. Radiol Clin North Am 15:297–308, 1977.

E. T. Creagan
S. Frytak
P. Pairolero
R. G. Hahn
J. R. Muhm

Division of Medical Oncology
and Departments of Thoracic and Cardiovascular Surgery and Diagnostic Roentgenology
Mayo Clinic
Rochester, Minn 55901

June 13, 1978

Prolonged Complete Remission in Malignant Fibrous Histiocytoma Treated With Chemotherapy[1]

Malignant fibrous histiocytoma is a rare soft tissue sarcoma of probable histiocytic origin (1). In the past, chemotherapy for most soft tissue sarcomas in adults has been only palliative at best. Occasional case reports have been published which noted prolonged complete remissions (CR) of inoperable sarcomas treated with chemotherapy (2).

We report here on a patient with malignant fibrous histiocytoma and neurofibromatosis who has remained in CR for 36 months after discontinuing chemotherapy. Results such as these point out the need for a positive attitude in the treatment of such patients.

CASE REPORT

A 45-year-old white man with known neurofibromatosis first noted a painful enlarging mass in the right inguinal region in February 1975. In March he was evaluated by a surgeon who noted a circular mass, 4 cm in diameter, located in the middle one third of the right inguinal canal. The mass was fixed to the underlying tissue. An excisional biopsy was carried out. Because of uncertainty about the pathologic diagnosis, the tissue was sent to the Armed Forces Institute of Pathology where the diagnosis of malignant fibrous histiocytoma was made (AFIP No. 1512020).

One month later, the patient was referred to West Virginia University Hospital because of a rapid recurrence of the lesion after the excision.

An examination at that time revealed a 9 × 7-cm mass in the right inguinal region. A repeat biopsy of the mass confirmed recurrence of the malignant fibrous histiocytoma. A search for metastases including whole-lung tomograms, a liver/spleen scan, and a bone scan was negative. The lesion was felt to be technically unresectable because of its extent and location in the inguinal canal.

The patient was treated with megavoltage radiation therapy at a dose of 2000 rads in ten fractions with a single anterior port to the right inguinal area. The tumor increased in size during the radiation therapy, and the patient began to deteriorate with anorexia and serious weight loss. Because the radiation had no effect it was discontinued, and systemic therapy consisting of adriamycin (25 mg/m^2) iv daily for 3 days every 3 weeks was instituted. After the second course of adriamycin, a CR was obtained. The adriamycin was continued and the patient received a total dose of 960 mg over 6 months. The drug was stopped at 550 mg/m^2. He received no therapy for the next 36 months and remains in CR for a total of 40 months.

DISCUSSION

Malignant fibrous histiocytoma is a rare soft tissue malignancy which constitutes 3%–4% of all

[1]Supported in part by Public Health Service grants CA-07757 and CA-19530 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

soft tissue sarcomas (3). The tumor may occur at any age but seems to be more prevalent in patients > 40 years old. It most commonly occurs in the soft tissues of the upper and lower extremities (4).

The increased incidence of soft tissue sarcomas in patients with neurofibromatosis is well known. The exact reason for this occurrence is speculative at this time. These tumors tend to be malignant neurilemoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma, and unclassifiable spindle cell type sarcomas (5,6). The fact that these patients have neurofibromatosis does not seem to affect the natural history of or recommended therapy for these sarcomas.

Recent advances in the chemotherapy for adult soft tissue sarcomas have been made with adriamycin, a highly active drug for these tumors (7). Unfortunately, most patients get only palliative benefit with chemotherapy.

This patient has had a long-duration CR after receiving adriamycin therapy. He had had radiation therapy prior to the adriamycin, and there may have been a synergistic effect on the tumor by these two modalities. However, he received only 2000 rads and tumor progression clearly occurred while the patient was receiving the radiation, so it is felt that chemotherapy alone was the successful treatment modality.

There are very little data available on the chemotherapy for malignant fibrous histiocytomas. The Southwest Oncology Group has the largest reported series. The combination of cyclophosphamide, vincristine, adriamycin, and DTIC or actinomycin D in 18 evaluable patients produced seven partial responders, for a response rate of 39%. The median duration of response was 10 months (range, 2–14 months). No complete responders were noted (3). In a review of adriamycin therapy in sarcomas, four patients with malignant fibrous histiocytomas were noted. Two patients had a slight response and two had progressive disease (7).

Our patient seems to be unique in having a prolonged CR of his inoperable malignant fibrous histiocytoma for 40+ months after treatment with adriamycin. We feel that the possibility of cure may be real in this patient.

REFERENCES

1. FU Y-S, GABBIANI G, KAYE GI, ET AL. Malignant soft tissue tumors of probable histiocytic origin (malignant fibrous histiocytomas): general considerations and electron microscopic and tissue culture studies. Cancer 35:176–198, 1975.
2. GOLDMAN RL, JONES SE, and HEUSINKVELD RS. Combination chemotherapy of metastatic malignant schwannoma with vincristine, adriamycin, cyclophosphamide, and imidazole carboxamide. Cancer 39:1955–1953, 1977.
3. LEITE C, GOODWIN JW, SINKOVICS JG, ET AL. Chemotherapy of malignant fibrous histiocytoma. A Southwest Oncology Group report. Cancer 40:2010–2014, 1977.
4. KEMPSON RL, and KYRIAKOS M. Fibroxanthosarcoma of the soft tissues. A type of malignant fibrous histiocytoma. Cancer 29: 961–976, 1972.
5. PRESTON FW, WALSH WS, and CLARKE TH. Cutaneous neurofibromatosis (Von Recklinghausen's disease): clinical manifestations and incidence of sarcoma in sixty-one male patients. Arch Surg 64:813–827, 1952.
6. D'AGOSTINO AN, SOULE EH, and MILLER RH. Sarcomas of the peripheral nerves and somatic soft tissues associated with multiple neurofibromatosis (Von Recklinghausen's disease). Cancer 16:1015–1027, 1963.
7. BENJAMIN RS, WIERNIK PH, and BACHUR NR. Adriamycin: a new effective agent in the therapy of disseminated sarcomas. Med Pediatr Oncol 1:63–76, 1975.

Wilbur B. Bassett[2]
Raymond B. Weiss*

Division of Medical Oncology
Department of Medicine
West Virginia University Hospital
Morgantown, WVa 26505

June 13, 1978

[2]Dr. Bassett is an American Cancer Society Fellow in Medical Oncology.

**Reprint requests to:* Dr. Raymond B. Weiss, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Landow Bldg, Rm C803, National Institutes of Health, Bethesda, Md 20014.

Use of Metronidazole and Other Nitroimidazole Drugs in Cancer Therapy

In a recent paper, Frytak et al (1) reported on a phase II study of metronidazole therapy for advanced colorectal carcinoma. They concluded that high-dose metronidazole would not seem to have any role in the treatment of this form of cancer. We should like to make some comments on why we believe the observations of Frytak et al may be misleading.

The nitroimidazole drugs, metronidazole and misonidazole, are undergoing trials as hypoxic cell sensitizers in radiotherapy. An additional property of these drugs is that they are differentially toxic towards hypoxic cells and it is this property that has suggested that these compounds might have a chemotherapeutic potential (2).

There is now little doubt that hypoxic cells constitute a radiation-resistant subpopulation in tumors and are therefore a limiting factor in tumor control by radiotherapy (3). Also, there is some evidence to suggest that these hypoxic cells may be a factor in the resistance of some solid tumors to chemotherapy. There are two likely reasons for this: (*a*)

potentially clonogenic cells rendered hypoxic may become noncycling or at least have their progression slowed down, and (*b*) hypoxic cells tend to be located near necrotic, or poorly vascularized, regions in tumors. Therefore, it is these cells that are less likely to be accessible to standard chemotherapeutic agents.

It has been shown that animal tumors as small as 6.0 mm^3 can contain hypoxic cells (4). Therefore, if we assume, as seems likely, that the tumors treated by Frytak et al consist of two cell populations (ie, those cells which are hypoxic and those which are in an aerobic state), we would suggest that, at the dose of metronidazole used, only hypoxic cells would be susceptible to cell killing. This, in isolation, would be of little value since the proliferating aerobic fraction of cells would be left unaffected. In this way, little or no subjective clinical improvement would be apparent.

In conclusion, we suggest that it is quite likely that drugs like metronidazole or misonidazole have a role to play in cancer treatment, but only if their use is accompanied by a modality such as radiation or cycle specific drugs which will remove the rapidly proliferating aerobic cells.

REFERENCES

1. FRYTAK S, MOERTEL CG, CHILDS DS, ET AL. Phase II study of metronidazole therapy for advanced colorectal carcinoma. Cancer Treat Rep 62:483–485, 1978.
2. ADAMS GE, FOWLER JF, and WARDMAN P, eds. Hypoxic cell sensitizers in radiobiology and radiotherapy. Br J Cancer (suppl 3) 37:1–321, 1978.
3. DISCHE S. Radiosensitizers in the treatment of squamous cell carcinoma of the head and neck. Clin Otolaryngol 2:403–409, 1977.
4. STANLEY JA, SHIPLEY WV, and STEEL GG. Influence of tumour size on hypoxic fraction and therapeutic sensitivity of Lewis lung tumour. Br J Cancer 36:105–113, 1977.

I. J. Stratford
G. E. Adams

Institute of Cancer Research
Royal Marsden Hospital
Clifton Ave
Sutton, Surrey SM2 5PX England

July 17, 1978

Combination of Constant-Infusion 5-Fluorouracil, Methyl-CCNU, Mitomycin C, and Vincristine in Advanced Colorectal Carcinoma

The combination of methyl-CCNU and 5-fluorouracil (5-FU) with or without vincristine has shown slightly higher response rates in advanced colorectal adenocarcinoma than have been observed with single agents or with other combinations (1–3). We decided to study a variant of this combination in patients with advanced colorectal adenocarcinoma by using the combination of prolonged-infusion 5-FU, the nitrosourea methyl-CCNU, vincristine, and the antibiotic mitomycin C. Although not adequately compared in a randomized study, it appears that the continuous slow infusion of 5-FU does not have the myelotoxicity of the bolus loading course of rapid-injection 5-FU (4,5). Fewer than 10% of the patients experience myelotoxicity, but gastrointestinal toxicity is more frequent. The response rates appear equivalent. Mitomycin C is also an active drug and appears to be the most active agent next to 5-FU and the nitrosoureas. Like the nitrosoureas, mitomycin C produces delayed myelosuppression, but if given as a single intermittent iv dose at 6–8-week intervals, there is reasonable time for marrow recovery. The combination of methyl-CCNU and mitomycin C would be expected to produce myelotoxicity at about the same time. Therefore, doses of each drug were necessarily reduced below the usual single-drug dose. Full doses of slow-infusion 5-FU and vincristine were combined with mitomycin C and methyl-CCNU for previously untreated patients with advanced measurable colorectal adenocarcinoma.

PATIENTS AND METHODS

Nineteen previously untreated patients were treated with the regimen as shown in figure 1. The primary sites of evaluable disease included liver (eight patients), lung (seven patients), lymph nodes (three patients), and skin (one patient). Continuous-infusion 5-FU was given for 3–5 days depending on the severity of gastrointestinal toxicity as monitored by degree of stomatitis and/or diarrhea. In only four patients was it necessary to stop the 5-FU at 3 days. Doses of methyl-CCNU and mitomycin C were adjusted in successive courses according to hematologic toxicity experienced during the previous course. 5-FU given on Day 21 was not attenuated unless the wbc count was $< 1500/mm^3$ or the platelet count was $< 50{,}000/mm^3$ (two occasions). Doses were withheld until these levels were achieved. Courses were repeated every 6–7 weeks.

Partial remission was defined as a $> 50\%$ reduction in the product of the perpendicular measurements of all sites of measurable disease, other than the liver, without evidence of tumor progression at new sites. In those patients with primary liver involvement, the liver edge was clearly palpable ≥ 5 cm below the xiphoid process or costal margins with quiet respiration. Objective response was defined as $\geq 30\%$ decrease (partial response) in the sum of the

(a) 5-FU, 30 mg/kg/day iv continuous infusion, Days 1-5 and Days 21-25

(b) Methyl-CCNU, 100 mg/m^2 orally, Day 1

(c) Mitomycin C, 6 mg/m^2 iv, Day 1

(d) Vincristine, 1 mg/m^2 iv, Day 1

(e) Repeat entire course every 6 wks

FIGURE 1.—Chemotherapy for metastatic adenocarcinoma of the colon and rectum.

measurements below the xiphoid process and both costal margins at the midclavicular lines. Stabilization was defined as either < 50% reduction in the size of the lesions or no increase in their size for ≥ 6 weeks.

RESULTS

No patient had a total regression of measurable disease. Two of 19 patients receiving the four-drug combination had a partial response (11%) as measured in liver and skin with durations of 8 and 12+ months. Four patients remained stable for 3, 4, 4, and 5 months. The responding or stable patients received a median of two and one-half courses of chemotherapy; nonresponders received a median of one and one-half courses. Median survival for all patients was 23 weeks.

Moderate to severe myelosuppression was the primary toxic effect observed in all patients (15 of 19 patients with platelet nadirs < 50,000/mm^3 and 16 of 19 with wbc nadirs < 1500/mm^3). Chemotherapy was associated with nausea and vomiting in 14 patients (73%). Gastrointestinal toxicity from infusional 5-FU occurred in eight patients (42%) in the form of moderate stomatitis and diarrhea.

DISCUSSION

Despite combining similar although not identical drugs, we were unable to achieve a similar response rate in previously untreated patients as reported for combinations of methyl-CCNU and 5-FU with or without vincristine.

This series of patients was heavily weighted with patients with pulmonary metastasis as the only objective measurement (seven of 19 patients). Pulmonary metastases are known to be less responsive to 5-FU, and this may be one reason for the lack of efficacy observed. The combination of methyl-CCNU plus mitomycin C may be another factor explaining the low response rate. The overlapping myelotoxicity of these drugs required lowering the dose of each, which might have decreased their expected efficacy. This treatment regimen, as applied in this study, cannot be recommended for future investigation.

REFERENCES

1. FALKSON G, and FALKSON HC. Fluorouracil, methyl-CCNU, and vincristine in cancer of the colon. Cancer 38:1468–1470, 1976.
2. MOERTEL CG, SCHUTT AJ, HAHN RG, ET AL. Brief communication: Therapy of advanced colorectal cancer with a combination of 5-fluorouracil, methyl-1-3-*cis*(2-chloroethyl)-1-nitrosourea, and vincristine. J Natl Cancer Inst 54:69–71, 1975.
3. MACDONALD JS, KISNER DF, SMYTHE T, ET AL. 5-Fluorouracil (5-FU), methyl-CCNU, and vincristine in the treatment of advanced colorectal cancer: phase II study utilizing weekly 5-FU. Cancer Treat Rep 60:1597–1600, 1976.
4. EINHORN LH, FURNAS BE, BOND WH, ET AL. Combination chemotherapy with 5-day continuous infusion 5-fluorouracil (5-FU) and methyl-CCNU in disseminated enteric adenocarcinoma. Proc Am Assoc Cancer Res and ASCO 16:19, 1975.
5. SEIFERT P, BAKER LH, REED ML, ET AL. Comparison of continuously infused 5-fluorouracil with bolus injection in treatment of patients with colorectal adenocarcinoma. Cancer 36:123–128, 1975.

F. Anthony Greco
Ronald L. Richardson
Susan F. Schulman
Robert K. Oldham

Division of Oncology
Department of Medicine
Vanderbilt University Medical Center
Nashville, Tenn 37232

June 26, 1978

Unusual Neuromuscular Toxicity After Treatment of Bone Sarcomas With Adriamycin, Vincristine, and Cyclophosphamide

Only a few instances of convulsive episodes following antiproliferative drug treatment have been reported. Gams and Carpenter (1) reported two patients with malignant melanoma and one with pleomorphic sarcoma in whom seizures developed (possibly due to cerebral hemorrhage) after treatment with adriamycin (ADM) and DTIC. Rosso et al (2) described a patient with retroperitoneal fibromyxosarcoma who developed transitory generalized seizures without loss of consciousness after treatment with DTIC, ADM, and vincristine (VCR).

Convulsive complications after cancer chemotherapy must be considered rare phenomena. Porter and Jick (3), in their study on drug-induced anaphylaxis, convulsion, deafness, and extrapyramidal symptoms in selected medical wards in the US, reported drug-induced convulsions in 26 of 32,812 patients receiving various drug treatments. In no patients were complications produced by antiprolif-

erative drugs. Weiss et al (4), in their review on the neurotoxicity of antineoplastic drugs, reported very few instances of seizures or muscle spasms following cancer chemotherapy with VCR and intracarotid mechlorethamine.

In the present paper, two case reports of patients with bone sarcoma who developed multiple muscle spasms and rigidity following chemotherapy are presented.

CASE REPORTS

Patient 1.—A 16-year-old white male developed an "osteosarcoma" in the proximal epiphysis of his right tibia. After amputation, no abnormal clinical or laboratory data were detected, and adjuvant chemotherapy was started. This patient received 1 mg/kg of ADM on Days 1 and 2. Two hours after the second administration, trismus, muscle spasms, and rigidity occurred. Thiocolchicoside (4 mg iv) and hydrocortisone (1 g iv) were administered. The symptoms gradually disappeared but a convulsive generalized spasm developed again 3 hours later. Since this spasm was similar in clinical pattern to tetanus, antitetanus immunoglobulins and muscle-relaxant drugs were administered. The symptoms gradually disappeared. Fifteen days later, 1 mg/m^2 of VCR and 10 mg/kg of cyclophosphamide (CP) were given, and 6 hours later, similar nervous symptoms developed which required muscle-relaxant drugs. After a few days, a placebo administration was given and was not followed by any symptoms. One month later, 1 mg/kg of ADM, and 2 months later, 1 mg/m^2 of VCR produced analogous clinical patterns. In the following 10 months, the patient received five courses of chemotherapy which were not followed by any symptoms, while a sixth course of ADM and CP resulted in muscle spasms and rigidity. Presently, the patient is in good clinical condition.

Patient 2.—A 17-year-old white male developed Ewing's sarcoma in the right humerus. After a biopsy, he was submitted to radiation therapy (6000 rads), and 2 months later he received chemotherapy. This patient received 1 mg/kg of ADM on Day 1 and 1 mg/m^2 of VCR on Day 2. Five hours after the second drug, generalized convulsive spasms, rigidity, and oculogyric movements developed. Diazepam was administered and symptoms gradually disappeared. One month later, a second course of chemotherapy was begun consisting of 1 mg/kg of ADM on Day 1 and 10 mg/kg of CP on Day 2. Seven hours after the second drug, an analogous clinical pattern developed. A subsequent placebo administration was not followed by any symptoms. A third course of ADM alone was given without consequences while an identical fourth course produced muscle spasms and rigidity. In the successive months, the patient received four more courses of chemotherapy, including ADM, VCR, CP, and methotrexate, without the appearance of any neurologic or muscular symptoms. The patient is still in good clinical condition.

In both patients, laboratory tests showed that a moderate increase of serum creatine phosphokinase followed convulsive episodes while other laboratory examinations including electroencephalograms, brain scanning, serum and rbc cholinesterases, electrolytes, and various serum enzymes showed completely negative results.

During the first course of treatment, both patients received, as is the routine in our hospital, allopurinol (300 mg/day) and a phenothiazine (prochlorperazine, 20 mg im 1 hour before treatment). Subsequent courses were given without any drug other than the antiproliferative ones. In addition, both patients received allopurinol and phenothiazines alone during an interval between two courses of therapy without any neurologic symptoms occurring.

DISCUSSION

The unusual complications of cancer chemotherapy that occurred in the above patients are difficult to explain. The characteristics the two patients have in common, age, sex, and malignant bone disease, all lead to the hypothesis that a biochemical-genetic peculiarity, as yet undetermined, could be the basis for these unusual toxic effects of cancer chemotherapy.

REFERENCES

1. GAMS RA, and CARPENTER JT. Central nervous system complications after combination treatment with adriamycin (NSC-123127) and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388). Cancer Chemother Rep 58:753–754, 1974.
2. ROSSO R, BREMA F, and PORCILE G. Description of a case of acute neurological toxicity after DTIC-adriamycin-vincristine therapy. Tumori 61:313–314, 1975.
3. PORTER J, and JICK H. Drug-induced anaphylaxis, convulsions, deafness, and extrapyramidal symptoms. Lancet 1:587–588, 1977.
4. WEISS HD, WALKER MD, and WIERNIK PH. Neurotoxicity of commonly used antineoplastic agents. N Engl J Med 291:127–133, 1974.

Efisio Sulis

Divisione Medica I
Ospedale Oncologico "A. Businco"
Via Jenner I-09100
Cagliari, Italy

July 17, 1978

ANNOUNCEMENTS

Conference on "Combined Modalities: Chemotherapy/Radiotherapy"

Hilton Head, South Carolina, November 15–18, 1978

The Committee for Radiation Oncology Studies, in conjunction with members of the Radiation Study Section and leading oncologists and with the support of the National Cancer Institute, is sponsoring a conference on the interaction of chemotherapy and radiotherapy. The meeting will focus on experimental results of laboratory and clinical studies of the interaction of selected cancer chemotherapeutic agents and radiation. The emphasis will be on the response of both normal and tumor tissues.

Attendance will be limited to people working in the field, certain invited speakers and participants, and those investigators who submit abstracts that are accepted by the organizing committee. It is intended to be a workshop-type meeting and abstracts will be presented either in open session or as poster-scientific exhibit sessions.

The research to be reported will include pharmacology, biochemistry, and basic chemical effects of the specific compounds, in vitro results with interaction of drug and radiation, in vivo normal tissue response to drug and radiation, and current clinical trial information. The following drugs are of special interest: bleomycin, adriamycin, cyclophosphamide, *cis*-platinum, and nitrosoureas. Papers on other agents will be considered for a session on other interesting and new agents.

For further details on the meeting write to: Theodore L. Phillips, MD, Chairman, Organizing Committee, Division of Radiation Oncology, University of California, M-330, San Francisco, Calif 94143.

Conference on "The Diagnosis and Management of the Complications of Cancer"

San Diego, California, February 19–23, 1979

A conference on "The Diagnosis and Management of the Complications of Cancer" will be presented at the Holiday Inn at the Embarcadero, San Diego, California, February 19–23, 1979.

This conference is equivalent to 32 hours of Category 1 CME credit. The registration fee is $280. *For further information, please write to:* Nomi Feldman, Conference Coordinator, Box 705, La Jolla, Calif 92038 (telephone: 714/459-0511).

Seminars in Oncology at Roswell Park Memorial Institute

Buffalo, New York

There will be a seminar on "Endocrinologic Aspects of Cancer" on December 7, 1978, organized by the Department of Medicine C at Roswell Park Memorial Institute. The chairperson is Dr. Avery Sandberg.

There will be a $25.00 registration fee. *For further information contact:* Ms. Claudia Lee, Cancer Control, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

*U.S. GOVERNMENT PRINTING OFFICE: 1978 260-909/6 1-3

If your address has changed and you wish to continue receiving this publication, please send your new address and the mailing label from this copy to:

Cancer Treatment Reports
National Cancer Institute
8300 Colesville Rd
Blair Bldg. Rm 3A05
Silver Spring MD 20910

If you do not wish to continue receiving this publication, please notify us at the address noted above and enclose the mailing label from this copy.

A publication of the National Cancer Institute

DHEW Publication (NIH) 78-22